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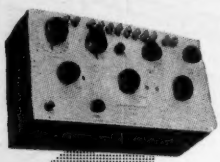

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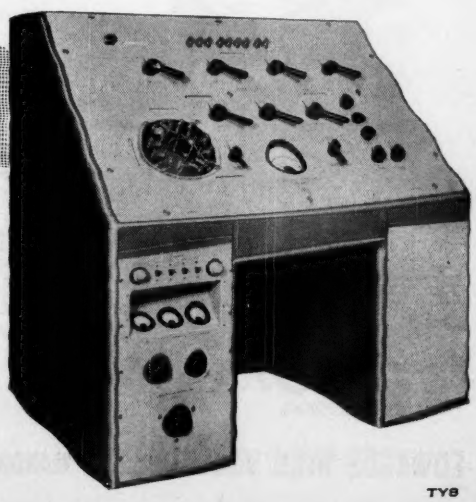

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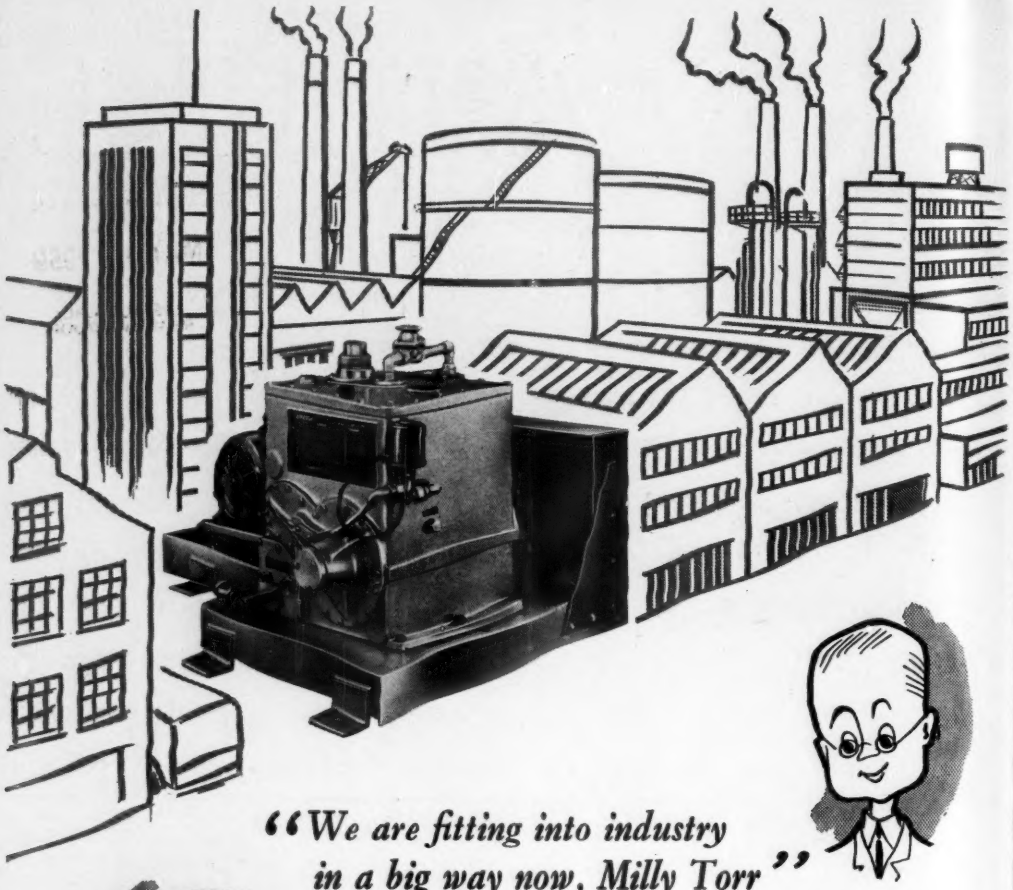

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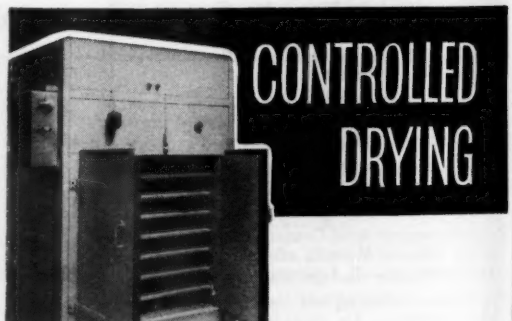

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THE RELATION OF EDUCATION TO RESEARCH

IN opening a debate on education in the House of Commons on January 22, the Minister of Education, Mr. Geoffrey Lloyd, stressed the need for maintaining a balance between the different steps on the educational ladder. He said little about the grammar schools, apart from a passing tribute to the way they had adapted themselves to the scientific age. The secondary modern schools had no long traditions to help them, but Mr. Lloyd said that in spite of handicaps some of them had considerable achievements to their credit, and 75 per cent of all children in some schools remained on after the compulsory age. Referring to the supply of teachers, Mr. Lloyd said that he hoped to make the training colleges much more like university institutions than many of them had been in the past, and that if it was clear that the 50 per cent expansion proposed by the Government was not enough, the Government would re-consider the expansion programme. There was no reference specifically to science teaching, but it has been stated that £2 million a year is being spent on laboratory buildings at maintained secondary schools, though without much more help the position at this critical point may well deteriorate rather than improve, as Dr. K. Hutton's recent address at the North of England Education Conference indicated.

In replying on the debate, the Parliamentary Secretary to the Ministry of Education, Sir Edward Boyle, pointed out that while the number of children in primary schools had fallen by 150,000–200,000 in the past two years, 200,000 extra places have been provided. Primary schools themselves will benefit in three ways from the Government's proposals for secondary education. Removal of senior children from all-age schools will leave more space for the younger children; resources are to be found during the five-year period both for building new primary schools in new housing areas and for completely replacing some of the worst primary school buildings; and a substantial part of the resources available for minor projects (costing less than £20,000) will be devoted to improving primary schools. As regards the reduction of all classes, junior and senior, to a maximum of thirty, Sir Edward Boyle pointed out that this would require a further 110,000 teachers over and above the present 260,000, and an increase of this scale is impracticable during the next few years. Finally, he repeated the assurance in the White Paper that there would be no attempt at a uniform pattern of secondary education. Whatever the best solution may be in new housing areas or sparsely populated areas, in Mr. Lloyd's view it would be wrong so to organize secondary education in the big cities of Britain as to leave parents no freedom of choice and to uproot good established schools, especially grammar schools, which are already doing first-class work.

Almost the only reference to expenditure in the debate was that of Mr. J. C. Jennings, who pointed out that the Government is already committed to £300 million for school building in the next five years, to £15 million for training colleges, £100 million for technological education and to £95 million between 1957 and 1963 for universities, and it is expected that the total figure of approaching £800 million for the present year would reach £1,000 million by 1963–65. Since then, the Minister of Education has given further details of the training college programme, which will include about seventy projects and is inspired by five principles: a considerable increase in the average size of the colleges, so that 40 per cent will have more than 400 places and provide 60 per cent of the total intake; the colleges will be made much more like university institutions than in the past; there will be a sensible distribution of the new places throughout England and Wales, with particular attention to areas with a serious shortage of teachers; special emphasis is being given to the training of more teachers of science and mathematics, and of certain other practical subjects; and the number of colleges catering for both men and women will be more than doubled.

The prospective supply of staff was one factor which has led the Minister to limit the current programme to 12,000 places, but Mr. Lloyd has already told the House of Commons that he is prepared to extend the programme if it appears that this 50 per cent expansion is insufficient, though he indicated that it would probably be wiser to extend the programme for a year or two after 1962 than to attempt more before then. On February 9 the Chancellor of the Exchequer announced that he proposed to ask Parliament to increase by £2,550,000 the recurrent grant of £34,350,000 already proposed for the universities for 1959–60, and to increase those for 1960–61 (£36,750,000) and 1961–62 (£39,500,000) by £2,660,000 and £2,720,000, respectively. The Department of Scientific and Industrial Research, moreover, is now prepared occasionally to recommend grants for special researches for periods up to seven years initially, instead of five years, as hitherto, and its new policy may well tend to increase the total allocations to the universities, even if the actual awards are earmarked for research. Furthermore, some of the evidence submitted to the Anderson Committee on Grants to Students, notably the recommendations from the National Union of Students that awards should be made without reference to parental income, would, if accepted, further increase the national expenditure on university education by perhaps £1·5 million a year.

Apart from its failure to consider expenditure and the critical issue of science teaching, the debate was disappointing in that secondary education was not properly considered either in relation to the educational system of Britain as a whole or, apart from the stress laid by both Ministers on the need to consider the individual boy or girl, to the demand for talent. It should be almost a corollary of providing equality of opportunity and freedom for the

gifted individual student to develop his or her ability to the full that there should be some relation between the scale of public education and the opportunities which the nation can provide for trained man-power. Notably the debate was characterized by lack of appreciation of the interdependence of the various levels of education, and of the way in which expenditure at one stage might limit expenditure at others, quite apart from considerations of available teachers or other resources.

Such considerations should determine Britain's expenditure on university and technical education, and the form which university expansion should take. Dr. B. V. Bowden has urged that we need more and bigger schools and more and bigger universities if we are to develop higher education sufficiently to meet Britain's needs as a great manufacturing and trading country. He has pointed out, too, that industry has a large part to play in developing and financing this expansion, and that unless industry prepares now, the larger number of graduates in technology and engineering who will be leaving the universities and colleges of technology in a few years time will be unable to complete their education. Moreover, such co-operation is not entirely one-sided. In Dr. Bowden's view, British universities do not make sufficient use of the facilities for research which are available in industry or through the Department of Scientific and Industrial Research.

This is a particular aspect of the organization in Britain for research, and it is one to which due attention should be paid in considering the functions of that Department's own research stations and of the research associations. There are dangers in the isolation of such institutions from academic life, as there are in the isolation of the universities themselves; and the repercussions between research and teaching are also complex and merit more attention. Apart from the question whether students in general should be encouraged to take up research unless they contemplate such a career and evince aptitude for research, there are questions as to the bearing of research on effective teaching, particularly in relation to facilities and the technical assistance available to the teacher who is also occupied in research.

All this is related, too, to the question of the size and distribution of universities in Britain as well as their number. The discussion on the size of universities and university departments at the Conference of the Universities of the United Kingdom last December showed that we are as yet far from agreement as to the right solution of the problem of university expansion. Sir Patrick Linstead argued cogently that it is important at such a time of rapid expansion to consolidate existing institutions rather than found new ones, and the argument that proper weight must be given to the efficient size of departments and of faculties has the more force when it is essential to ensure the maximum use of available resources in finance and in teaching staff. Sir Patrick considers that a university department in subjects which are rapidly expanding and sub-dividing should have at least 150–200 students, and while he does not favour expansion of non-collegiate universities beyond about 4,500 students, he thinks that an arts faculty should contain at least 1,000, a science faculty 600 and a faculty of applied science 800 students.

Sir Patrick believes that the principles he puts forward, if applied to the existing universities of Britain, including the projected University of Sussex, would provide sufficient space for all but 5,000 of 124,000 students visualized by 1965, and that this deficiency could be met by founding one new university, for example, at Norwich, and developing three or four new faculties of technology at existing universities. Prof. E. Devons thinks that the problem of size requires rather more study, and like Lord Simon in a recent article in the *Universities Quarterly*, leans towards a comparative study of university administration and organization, without, however, suggesting that a Royal Commission is needed. Lord Simon, it is true, suggested that numerous other problems, such as the factors determining university expansion, the allocation and control of grants by the University Grants Committee, the abolition of fees, the relations between research and teaching, merit independent examination.

Dr. R. Pedley, however, at the Universities Conference, favoured the establishment of several new universities largely because of the unequal contribution which a university can make to the quality of regional culture, and for this reason visualized new universities on Teeside, in mid-Lancashire, Coventry and the Home Counties. He also thinks that a further 46,000 places will be required by 1965, even if only 5 per cent of the pupils in comprehensive schools qualify for university entrance. He does not believe, moreover, that a scarcity of university teachers of high calibre is a sufficient reason for denying university education to those who might profit by it, and, while urging the desirability of a graduate teaching profession, he is prepared to accept a higher ratio of students to staff until 1970 than at present, provided those students had learned in school how to study and how to organize their lives.

As might have been expected, the Universities Conference showed awareness of the need for the educational system to be balanced; but that is very far from being fully recognized by Parliament as yet, nor has the Government taken any apparent steps to secure such a balance, even within whatever limits of expenditure it is prepared to propose. The Earl of Dundee, in replying for the Government on a notable debate on the youth services in the House of Lords on February 4, did indeed stress the importance of the contribution which the varied institutions of further education, including many voluntary bodies, have to make to the preservation of the permanent values in human character on which the stability of our way of life so largely depends. Unless such virtues as discipline and unselfishness, patriotism and loyalty and true religion are actively fostered, the best literary and scientific education in the world will not preserve the soul of a great country, and scientific progress and material achievement may come to naught.

The problems of educational expansion are indeed complex and varied, but it is not difficult to discern the correct priorities at any moment, though those priorities will themselves change from time to time. What is of first importance at present is that policy should be determined not by political doctrine but by educational needs and experience. There is indeed much that is common ground between the major political parties, and ample room for experiment without disturbing the functioning of existing institutions by introducing political uncertainty. An all-party approach would likewise assist to make available the greater resources Britain clearly needs to allocate to education, and to secure the most effective distribution of those resources. The task at any level is too immense for us to look for success except by co-operation on a much greater scale than in the past, and to promote the appropriate atmosphere of co-operation is a first duty of every responsible citizen. In such an atmosphere it will be the easier to resolve wisely the inevitable clashes between sectional and national views, and to secure the agreement to allocate special effort or resources at whatever point, such as the training of teachers of science, the improvement of school laboratories, or the expansion of technical colleges, that may at the moment be most needed, or to take the less spectacular measures designed to avoid waste of talent and resources and ensure that the country gets full value from its mounting expenditure on education.

SOCIAL POLICIES AND SOCIAL CHANGE

Essays on 'The Welfare State'

By Prof. Richard M. Titmuss. Pp. 232. (London: George Allen and Unwin, Ltd., 1958.) 20*s*. net.

IN the course of the first of this series of essays Prof. R. M. Titmuss comments that it was characteristic of Prof. R. H. Tawney that he constantly reminded his students that "the most important thing about a man is what he takes for granted". It is equally characteristic of Prof. Titmuss that he makes it impossible for his readers to go on taking for granted those presuppositions about social policies and institutions which are all too easily accepted and acted upon by so many people. Whether he is writing about the differing provisions made for income maintenance in old age, about the position of women in modern society, about medical care under the National Health Service, he is careful to examine the relevant data afresh, often bringing to light new or little-known information in the process. The resultant discussions are always stimulating and illuminating. Sometimes they are disconcertingly so, and whether we accept his final conclusions or no, Prof. Titmuss certainly challenges us to think again about the subjects under discussion.

The topics covered in this collection of essays are varied and at first sight disparate, but, as is pointed out on the fly-leaf, two broad themes unite them—changes in social structure and developments in social policy, and it is one of the chief merits of Prof. Titmuss's whole approach that he relates the two processes in a way seldom attempted either by academic sociologists or students and practitioners of social administration. For example, pension policies are considered in the context of population change, and current problems and practices of the National Health Service are related both to the scientific revolution in medicine which began in the late 1930's and to the psychological and social factors affecting the individual's and society's attitude towards health, disease and medical care. The relationship between institutions and policies is similarly emphasized in those essays which take as their starting point a social institution. For example, in the essay on "Industrialization and the Family", Prof. Titmuss shows that, on one hand, problems of human relations in industry cannot be solved within industry itself, and on the other, that the family does not function in a social vacuum. Rather is it profoundly affected by the forces of industrialization, and in this context we can see the social services in "a variety of stabilizing, preventive and protective roles".

Not only do these essays differ in subject-matter, they are based on lectures given at different times and places to audiences of different background and outlook. The first was delivered in 1951 at the London School of Economics, whereas those on the National Health Service were addressed to an American audience at Yale in 1957. This does not destroy the underlying unity, but it does mean that each essay is more or less self-contained, and the book as a whole, valuable and stimulating as it is, is but a foreshadowing of what Prof. Titmuss might achieve had he the leisure and opportunity to produce a major work dealing with the broader aspects of social policy, to the study of which he brings such depth of insight and breadth of vision. M. P. HALL

THE QUINTESSENCE OF PARACELSUS

Paracelsus

An Introduction to Philosophical Medicine in the Era of the Renaissance. By Walter Pagel. Pp. xii+368. (Basel and New York: S. Karger, 1958.) 70 Swiss francs.

PROBABLY no other figure in the history of medicine and science offers greater difficulties to our understanding than Paracelsus. Probably none of the numerous books on Paracelsus is more helpful in forming an objective and clear appreciation of Paracelsus than this new book by Dr. Pagel.

The author achieves this goal by concentrating on the philosophy of Paracelsus, which sees cosmology, theology, natural philosophy and medicine in the light of analogies and correspondences between the macrocosm and the microcosm, man. Naturalism is with Paracelsus the search for divine signs. He is, therefore, with all his protoscientific ideas and achievements, not a scientist in the modern sense.

The author analyses in detail the Paracelsian concepts of 'experience', astrology, time, the elements, the famous three principles (sulphur, salt, mercury) and quintessence. There is a certain contradiction between the microcosmic dilution of man and the idea of specificity, equally important to Paracelsus, and embodied in the archeus or archei. 'Imagination' provides man with great spiritual power.

The medicine of Paracelsus is but an application of these general ideas. Paracelsus regarded diseases as specific fruits of the human body in the same fashion

in which the metals were specific fruits of the earth. In spite of his chemical pathology Paracelsus tends toward localizing disease on his firmament of organs. But disease is essentially the result of an extraneous force, an invader, a seed. This specific agent has to be treated etiologically by specific remedies (ancient therapy was non-specific), which are often chemicals. Paracelsus paid particular attention to diseases due to tartar, fought the doctrine of catarrh, and developed a new theory of plague. He began to replace medieval uroscopy by quantitative and chemical analysis of urine. The author gives a long list of his practical achievements in medicine, always combined with wild speculations (antiseptic principles, syphilis, mercury as a diuretic, hydrotelluric theory of goitre, balneology, miners' diseases).

Dr. Pagel then analyses with wonderful scholarship and great penetration the sources of Paracelsus: gnosticism, the Cabbala, neoplatonism, Avicebron, Ramon, Lull, Arnuld of Villanova, the alchemists John de Rupescissa, Reuchlin, and Agrippa of Nettesheym. He also discusses in detail Paracelsus's great critic, Erastus, and his moderate defender, Daniel Sennert.

Paracelsus was, according to the author, an able chemist, and his medicine has elements of what was to become modern medicine. But his chemistry and his medicine were but parts of his symbolistic, decidedly unscientific cosmology and philosophy. He produced scientific results from a non-scientific world of motives and thoughts. He offered little which in principle cannot be found with his medieval predecessors, but he integrated all these elements into a new doctrine of medicine and natural philosophy. Without Paracelsus there would have been no iatrochemistry. He was a 'Magus' who forged a new synthesis from personal experience.

I do not hesitate to rate this book as the most valuable in the whole extensive literature on Paracelsus from the point of documentation as well as judgment. It is a high point in the author's long and distinguished career as a medical historian, which began with his work on van Helmont in the late 1920's and continued the great accomplishments of his father, Julius Pagel. It avoids equally the nebulous enthusiasm and the embittered hostility encountered so often in writings on Paracelsus. Dr. Pagel unites an unqualified attachment to modern science with a profound feeling for and understanding of mystic and magic philosophy. This rare combination enables him to be just and to give us the quintessence of Paracelsus. ERWIN H. ACKERKNECHT

MEDICINE IN INDUSTRY

Industrial Hygiene and Toxicology

Edited by Frank A. Patty. Vol. 1: General Principles. Second, revised edition. Pp. xxvii+830. (New York: Interscience Publishers, Inc.; London: Interscience Publishers, Ltd., 1958.) 17.50 dollars.

THE first edition of Patty's "Industrial Hygiene and Toxicology" was published in 1948 in two volumes. The second edition is to be in three volumes, of which the first is now ready. It is not possible to assess the whole work until it finally appears, but, according to the preface to the second edition, much of the new matter has been placed in the first volume. The main changes are to be found in the treatment of such subjects as noise, heat, ionizing radiations, human engineering and industrial safety, sanitation, air pollution and illumination. Additional chapters on air conditioning, air cleaning, and heat control in the hot industries are included, as well as separate chapters on illumination and industrial sanitation. Dr. O. A. Sander contributes a revised chapter on the pulmonary dust diseases, Dr. L. Schwartz and Dr. Carey McCord bring up to date their chapters on the industrial dermatoses and the visible marks of occupation, respectively. The editor, Mr. Frank Patty, is responsible for four chapters on such subjects as the industrial hygiene survey, the mode of entry and action of toxic materials and sampling and analysis of atmospheric contaminants, in addition to his introductory chapter on industrial hygiene. This chapter calls for some comment, because Mr. Patty seems to have the idea that analyses of the contaminants of factory atmospheres did not begin in Great Britain until the formation of the Occupational Hygiene Society in April 1953, and that until that date medical control and not engineering control of health risks had been used. The fact is that both methods of control have been used in Great Britain for many years. Nevertheless, industrial or occupational hygiene is a rapidly developing subject, and the industrial health engineer is a new importation from the United States.

The first edition of Mr. Patty's book was an ambitious, but rather uneven, attempt to bring all the facets of industrial hygiene within the same close and curtilage. It showed that the industrial health engineer has the freedom of engineering, chemistry, physics, biology, physiology and medicine, and speaks with equal authority on all of them. In this age of super-specialization, the health engineer is therefore a refreshing phenomenon. The engineer who writes with authority on clinical medicine and hæmatology, without the benefit of a medical training, is to be congratulated on his courage. No doctor with experience of industry has yet been bold enough to write and edit a book on engineering. But that state of affairs may yet arise, because the industrial doctor in Britain is obliged to make himself familiar with the intimate details of factory processes, and with the estimations of contaminants in the atmosphere and its engineering control. In the United States it is rare for the plant doctor to deal with such matters, which are left to the health engineer, or industrial hygienist. It seems to us in Britain that the American version of the health engineer is probably taking on too much responsibility for health matters. No doubt in the course of time the industrial hygienist will find his proper place. Mr. Patty himself shows that the position in the States is being slowly modified. "Industrial hygiene," he writes, "has successfully withstood abortive efforts at absorption by both safety engineering and medicine. It borders on each of those fields, but differs in technique from both, and one of its primary functions is to get these two groups to work hand in hand." He has little doubt that regular and more frequent medical examinations are a desirable way of discovering dangerous exposures, especially in the absence of competent industrial hygiene engineering and control; and he affirms that when the two methods are properly co-ordinated, the most dependable safeguard against harmful exposures is provided. He has yet to reach the logical conclusion that the major responsibility for health matters in industry rests with the medical profession, assisted, as it should be, by capable and knowledgeable industrial hygienists.

A. I. G. MCLAUGHLIN

RADIOACTIVE ISOTOPES IN BIOCHEMISTRY

Radioaktive Isotope in der Biochemie

Von Prof. Engelbert Broda. (Einzeldarstellungen aus dem Gesamtgebiet der Biochemie, Neue Folge, Band 3.) Pp. viii+326. (Wien: Verlag Franz Deuticke, 1958.) 294 schillings.

FEW techniques have had a more widespread or profound influence on modern biochemistry than the application of isotopes. Although the first experiments were carried out by Hevesy more than thirty-five years ago, many isotopes have been available for a much shorter time and the use of isotopes in biochemical research has increased remarkably in the past fifteen years, as shown in an instructive table in this book. During the same period there have been considerable improvements in the instruments required for radioactive tracer work and there is thus sufficient scope for a new book, despite the existence of several well-established works.

Prof. E. Broda of the University of Vienna has written a book which is intended to serve both as an introduction to the methods used in work with radioactive isotopes and as an outline of the major biochemical problems in which isotopes have been particularly useful. Thus, the first eight chapters of the book deal with the production of isotopes, the synthesis and biosynthesis of labelled compounds, the hazards involved in work with radioactive substances, precautionary and protective measures, and the measurement of radioactivity. The treatment is concise, easily intelligible and useful for those interested mainly in acquiring sufficient knowledge for applying radioisotopes to their own problems.

The next two chapters deal with analytical applications, such as the isotope dilution method, and with metabolic pools and turnover. Finally, there are five chapters on main problems (phosphorylation, photosynthesis, carbon dioxide fixation, the metabolism of carbohydrate, fat, protein and nucleic acids, the citric acid cycle, purine and pyrimidine biosynthesis) and one on special problems of metabolism (immunological problems, the biosynthesis of porphyrins, cholesterol and lignin). This last chapter also contains a very brief (five pages) and somewhat inadequate section on the metabolism of biologically active compounds such as vitamins, drugs and carcinogens.

Although the book has been deliberately restricted to radioactive isotopes, stable isotopes are fortunately not completely neglected. For example, double intramolecular labelling with deuterium and carbon-14 is briefly mentioned (p. 31) and the importance of the stable isotope nitrogen-15 is indicated on pp. 223-29. The only notable omission is the application of isotopes, both stable and radioactive, to studies of the mechanism of enzymic reactions.

The book is well produced and there are very few errors. In some cases, however, there are slightly misleading statements, as for example on p. 16 where it is suggested that oxygen exchanges between the two positions of a carboxyl group, which are, of course, equivalent because of the symmetry of the carboxylic anion. It is a little surprising to read that the basic outline of protein synthesis is still completely unknown (p. 216) and that carboxyl activation of amino-acids is regarded as merely probable (p. 221). Also on p. 221 there is some confusion between transpeptidation and *de novo* peptide bond synthesis. But these are perhaps only minor matters in a book primarily concerned with isotopes.

The literature references are remarkably up to date, many of them being to publications which appeared in 1957. It is to be hoped that an English translation of this useful book will be forthcoming.

H. R. V. ARNSTEIN

NUCLEAR ENGINEERING MONOGRAPHS

Nuclear Engineering Monographs

No. 1, Elementary Nuclear Physics. By W. K. Mansfield. Pp. viii+60. 10*s*. 6*d*. net. No. 2, Nuclear Reactor Theory. By J. J. Syrett. Pp. viii+80. 12*s*. 6*d*. net. No. 3, Reactor Heat Transfer. By W. B. Hall. Pp. viii+68. 10*s*. 6*d*. net. No. 4, Nuclear Reactor Shielding. By J. R. Harrison. Pp. viii+68. 10*s*. 6*d*. net. No. 5, Nuclear Reactor Control and Instrumentation. By J. H. Bowen and E. F. O. Masters. Pp. x+78. 12*s*. 6*d*. net. No. 6, Steam Cycles for Nuclear Power Plant. By W. R. Wootton. Pp. vii+66. 10*s*. 6*d*. net. (London: Temple Press, Ltd., 1958.)

THE first three books in this excellent series were published about a year ago, and the final three quite recently. The six fall into two classes: Nos. 1, 2 and 4 provide summaries of matter which is readily available in greater detail elsewhere; and Nos. 3, 5 and 6 provide summaries of matter either not previously published or else dispersed rather widely.

There is general agreement that nuclear engineering is more appropriately studied at postgraduate level. However, nuclear physics is a useful subject for inclusion in the final year of undergraduate syllabuses and many universities provide this type of course. Advanced physics text-books are inappropriate at this stage, but the first monograph dealing with the modern concept of the atom, radioactivity, nuclear reactions and the detection of nuclear radiations is at a suitable level.

A physicist or engineer dealing in great detail with reactor physics might prefer to by-pass the monograph on reactor theory and work directly on the more comprehensive standard texts. The design of a reactor core is essentially a compromise of many conflicting criteria and a large number of engineering designers need to know the form in which the physicist does his calculations and the probable significance of changes in core geometry. Mr. Syrett's concisely written book outlines the framework of this important subject, and the text is sufficiently brief to assist in absorbing the principles. Even when reading the more detailed text-books it is useful to have the monograph to hand to show the way through what might otherwise appear a very complex analysis. The monograph deals with the slowing down and diffusion of neutrons, lattice calculations and the calculation of critical size. The chapter on "Reactor Operation at Power" is a particularly good introduction to the subject.

The third monograph in the first class mentioned above is that on reactor shielding. The author introduces his book by saying that a great deal of experimental and theoretical work is being undertaken on the subject of shielding and that 'art' is being replaced by science. Existing texts on the subject are bulky and contain masses of data. The monograph is welcomed as providing a guide to the important considerations; it is divided into three

main sections, the first dealing with gamma-rays and their attenuation, the second with the neutron physics relevant to shield design, and the third with practical shield design.

The first monograph in the second group is that on heat transfer. Little had been published on reactor core heat transfer in Britain prior to the 1958 Geneva conference. This book is divided into two sections, and most experimenters will be sympathetic in the method of splitting as between fundamental work and work of immediate practical application. The heat-transfer specialist in nuclear power has the twin function of testing fuel-element cans under simulated reactor conditions to give exact design figures, and of carrying out inspired research work to provide the answers for subsequent stages in reactor development. The chapter on fundamentals sets out in a concise form the analytical approach, and describes the various analogies between heat and momentum transfer. The second chapter derives the usual design formulæ for channel heat transfer and pressure drop and ends with a comparison of gaseous coolants and a worked example. The final chapter considers such topics as liquid-metal heat transfer, fin efficiency and a comparison between liquid and gaseous coolants.

The monograph on reactor control and instrumentation is welcomed as providing a basis for later, more detailed, texts on these subjects. The incident at Windscale emphasized the need for comprehensive instrumentation in present reactor designs, and the lecture by Sir John Cockcroft in Tokyo last November mentioned two phenomena, positive moderator temperature coefficient and disturbances in the power distribution in a reactor due to local changes in xenon poisoning, with which the designers of the current stations have to cope. The present monograph must suffice until nearer the commissioning times of the Central Electricity Generating Board and the South of Scotland Electricity Board stations. The subject of control is a complex one ranging from neutron kinetics to the thermal response of a steam-raising unit. The monograph deals with neutron kinetics conventionally. The following chapter on temperature-stabilized reactors is important. As the authors mention, this type of analysis would in practice be carried out using an analogue computor. The numerical and graphical methods described do, however, show the trends. In later editions of these monographs, the final chapter of this book on instrumentation might well prove the basis for a seventh monograph, and then include health physics instrumentation.

The monograph on steam cycles consists of two main sections, one dealing with graphite-moderated, gas-cooled reactors, and the other with water-cooled, water-moderated reactors. It is worth remembering that the dual pressure cycle for the former type of reactor was first suggested in 1948. Since that time considerable effort has been expended in dealing with the number of variables involved. The essential tools are given in the appendixes, but the work involved is laborious. It is to be regretted that some co-operative effort was not made years ago to publish comprehensive graphs of the form given in this book. The section on water-cooled reactors deals with both pressurized-water and boiling-water types, and includes a short section on the use of a coal-, oil- or gas-fired superheater.

The editor of this series is to be congratulated in providing a number of stimulating monographs.

R. Vaux

LOW TEMPERATURES

Low Temperature Physics and Chemistry

Proceedings of the Fifth International Conference on Low Temperature Physics and Chemistry held at the University of Wisconsin, Madison, Wisconsin, August 26–31, 1957. Edited by Joseph R. Dillinger. Pp. xxv+676. (Madison, Wis.: University of Wisconsin Press, 1958.) 6 dollars.

Kältetechnik

Von Prof. Matts Bäckström. Zweite überarbeitete Auflage. Bearbeitet und ins Deutsche übertragen von Dr.-Ing. Eduard Emblik. Pp. xvi+656. (Karlsruhe: Verlag G. Braun; London: K. G. Heyden and Co., Ltd., 1957.) 56 D.M.; 98*s*.

INTERNATIONAL conferences on the progress of low-temperature physics are held bi-annually. The book, published by the University of Wisconsin Press and edited by Dr. J. R. Dillinger, contains the reports of such a conference held in Madison, Wisconsin, in 1957. The reports were furnished by the speakers and, since it was originally intended to bring out a simple stencilled version quickly, the figures are in many cases rough diagrams. Altogether, more than two hundred reports from all branches of low-temperature research are presented, and it is, of course, quite impossible to give even a rough survey of the contents.

The previous low-temperature conference was held in Paris in 1955 and much of the work reported in Wisconsin is necessarily a continuation of research discussed at the Paris meeting. There were a number of new developments in the field of nuclear orientation. The most outstanding of these are the experiments on the anisotropy of beta emission which provided the proof of the non-conservation of parity postulated by Yang and Lee. Another paper reported the successful orientation of nuclei by the method suggested by Pound in which use is made of the coupling between the lattice electric field and the nuclear quadrupole moment. It is unfortunate that a special lecture by Dr. N. Kurti on nuclear cooling has been published separately and is not included in the book.

An important advance in the theory of superconductivity was made by Bardeen, Cooper and Schrieffer, and a number of papers were read on experimental confirmations of this work. In particular the work on absorption of radiation at wavelengths between 0·1 and 1 mm. and that on the change of ultrasonic attenuation when a metal becomes superconductive deserve mention. The first reports were also submitted on a practical application of superconductivity which may lead to far-reaching developments. This is the use of superconductive switching elements in computors and in memory devices.

A certain amount of new information on liquid helium and especially on the behaviour of the light isotope is noted. Interesting advances have been made in the cryogenic use of the latter and it was regretted that Soviet scientists, who have done much work in this field, had not been able to attend.

Dr. Bäckström's book is a translation into German of the Swedish original. It deals with a great many aspects of refrigeration technique, mainly in the range of interest for food storage. It is not a text-book but rather an information manual which will be of interest to those specializing in this field.

K. Mendelssohn

The Measurement of Soil Properties in the Triaxial Test

By Dr. Alan W. Bishop and D. J. Henkel. Pp. viii+190. (London: Edward Arnold (Publishers), Ltd., 1957.) 70*s*. net.

THE Department of Soil Mechanics in the Imperial College of Science and Technology, London, has a standing in teaching and research second to none among schools of soil mechanics, and its students are drawn from all over the world. This is at once an assurance that the training given is valued and widely disseminated. Dr. A. W. Bishop is reader and Mr. D. J. Henkel lecturer in this Department, and to read their book is to be taken with firm direction through a course of instruction in their own laboratory; to be taught sufficient about the application of triaxial tests to appreciate their significance, and much of the art of making the tests and keeping them meaningful. The authors tell us they do not intend their book as a manual, presumably because they have limited themselves to describing the methods and theories which have been developed at the Imperial College, but though it may not be a manual, there is no doubt that for many readers it will form an adequate text-book. Its four parts deal with the practical application of test data; the detailed construction of apparatus; the standard tests for which the apparatus is intended, and various special tests for which it may be used.

The ground is covered thoroughly, and where points of theory or application are not presented, the relevant references to the literature are given. Particularly welcome are the sections concerning the determination of the pore pressure parameters *A* and *B*, which form the basis of the concept of shear strength introduced by Skempton and Bishop in 1954. The book is well produced and contains many diagrams, detailed drawings and photographs of apparatus. Soil-mechanics engineers will welcome it as a work of reference, but its price will cause a student to debate the need for adding it to his own bookshelf.

THOMAS WHITAKER

Clinical Hæmatology in Medical Practice

By G. C. de Gruchy. Pp. xii+620. (Oxford: Blackwell Scientific Publications; Springfield, Ill.: Charles C. Thomas, 1958.) 50*s*. net.

IN the best organized institutions the physician and hæmatologist work in close collaboration. The hæmatologist must know enough about the clinical state of the patient to know which tests are appropriate, and the physician must know enough about hæmatology to be able to interpret the results. Dr. de Gruchy's book provides just this information. It gives an accurate balanced account of hæmatological disorders with enough clinical details to enlighten the hæmatologist and enough about the interpretation of technique for the physician. It would be very easy for such a book to become unwieldy, but Dr. de Gruchy has avoided this by the total omission of technical detail and the free use of tabulation. The presentation is somewhat dogmatic and it is this which makes the book so easy to read. But, of course, it is never possible to be dogmatic and agree with all workers on every subject. For example, it is probable that workers with experience in the surgical treatment of hæmophilic patients would not consider that a transfusion of 20 ml. of fresh plasma per kgm. body-weight would be likely to control bleeding. However, such details are really irrelevant; anyone wishing to undertake some very specialized treatment would naturally consult original papers on the subject and would not rely exclusively on a book, the main object of which is the general education of physicians and clinical pathologists.

R. BIGGS

Clinical Enzymology

Edited by Dr. Gustav J. Martin. Pp. vii+241. (London: J. and A. Churchill, Ltd., 1958.) 42*s*. net.

ALTHOUGH clinical enzymology is not a new sphere of activity in medical science it is a subject that has been greatly fostered by the purification and crystallization of enzymes during recent years. From this has come a realization that enzymes can be usefully administered parenterally in many instances. This book begins with a discussion of protein biology and the chemistry and biochemistry of enzymes which are of clinical value. The diagnostic use and the therapeutic action of enzymes are also considered and the book concludes with a discussion of future possibilities. The parenteral administration of trypsin in particular has been of value in the treatment of conditions involving intravascular clotting, in the treatment of infected wounds, and in a wide range of infections generally. On the whole, the anti-inflammatory action of the enzyme is the most striking. Other enzymes which have been used parenterally include streptokinase, deoxyribonuclease, hyaluronidase, and cholinesterase, but for the value of the use of most of these by parenteral administration only fragmentary evidence exists. It can be said without hesitation, however, that the medical use of parenteral enzymes is bound to grow and there is no doubt that clinical enzymology has come to stay.

F. G. YOUNG

Scientific and Learned Societies of Great Britain

A Handbook compiled from Official Sources. 59th edition. Pp. 215. (London: George Allen and Unwin, Ltd., 1958.) 35*s*. net.

THIS book, the third of its kind, derives from the "Yearbook of Scientific and Learned Societies", which ceased publication in 1939 after appearing almost continuously for more than fifty years. It is now edited by the British Council.

The greater part of the book consists of a list of nearly a thousand societies, ranging from the Royal Society down to the small local natural history societies, grouped under sixteen headings. Particulars of the objects, membership, meetings, publications, museum and library of each society are given. Some of the entries are of societies not strictly scientific, but inclusion is no doubt preferable to omission in compilations of this sort. The criterion is, in general, whether a society gives educational facilities to its members, or publishes original work.

There is also a list of the principal organizations conducting officially sponsored research, and a select bibliography on the organization of scientific research in Great Britain. In the former, universities, technical colleges and laboratories attached to commercial firms are, of course, not included; but neither are the industrial research organizations. These are officially sponsored since they receive Department of Scientific and Industrial Research grants, and they might usefully be brought into future editions.

BIOSYNTHESIS AND CONSTITUTION OF LIGNIN*

By Prof. KARL FREUDENBERG
University of Heidelberg

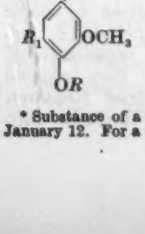
NEARLY forty years ago, by studying the kinetics of the degradation of cellulose, I obtained evidence that there exists a clear order in the large cellulose molecule, one glucose unit being bound to the next in the same way as in cellobiose[3]. This concept of a *uniform* chain, which was later adopted by H. Staudinger and W. N. Haworth, encouraged me to attack lignin, the other major wood constituent.

The problem was to decide whether lignin is a sort of molecular 'compost heap', or whether, like cellulose, it possesses an orderly structure. The second possibility was preferred as a working hypothesis.

However, lignin is a condensed system which cannot be hydrolysed, and the units from which it is built, though of similar types, are not identical. Analytical efforts to elucidate the lignin structure made but slow progress. It contains methoxyl[4], hydroxyl, and ether[5] groups, and can give 30 per cent of the theoretical yield of vanillin[6]. Formaldehyde[7] and *iso*-hemipinic acid (3-carboxy-4,5-dimethoxybenzoic acid)[8] are other products of degradation, and Hibbert[9] isolated a small amount of ketones by alcoholysis. In 1935, Holmberg[10] discovered that phenylcarbinol groups and their ethers are involved in the sulphite pulping process, and the Swedish school elucidated the specific nature of the phenylcarbinols responsible for this important reaction. Among these ethers are phenyl-coumarans, which had been proposed as constituents of lignin[11]. Despite these discoveries, it still remains impossible to construct a constitutional scheme for lignin from purely analytical information.

In 1875, Tiemann in Berlin cleared up the constitution of the glucoside coniferin (I) which is present in the cambium sap of conifers during the period of vegetation. In deciduous trees coniferin is found together with syringin. The present discussion is confined to coniferous wood.

Tiemann suggested that lignin and coniferin are related genetically[12], and this idea was used by Klason[13] throughout his work on lignin chemistry. H. Erdtman[14] showed that the product obtained many years ago by two French workers[15], who oxidized *iso*eugenol by means of mushroom oxidases, was in fact a phenylcoumaran similar in kind to that claimed to occur in lignin. This oxidation reaction provided a model for the formation of lignin, and in 1933 Erdtman[14] pointed out that lignin might be a dehydrogenation product of guaiacyl-propane derivatives with an oxidized side-chain.



(I) R and R_1 = H: coniferyl alcohol

(II) R = $C_6H_{11}O_5$; R_1 = H: coniferin

(III) R = H, R_1 = OCH_3: sinapinic alcohol

(IV) R = $C_6H_{11}O_5$; R_1 = OCH_3: syringin

* Substance of a lecture given in the University of Cambridge on January 12. For a recent survey of work on lignin, see refs. 1 and 2.

Fig. 1. Cross-section through a spruce sapling after administration of D-coniferin-β-^{14}CH

During the past eight years we have been studying the biosynthesis of lignin by the method of introducing various radioactive substances into spruce saplings. The needles of a small side twig are trimmed down, and the twig is allowed to suck up a solution containing the tracer[16]. It is found, when using radioactive D-coniferin, that the radioactivity is fixed within a few days in the region around the point of attachment of the branch, and radioactive lignin is obtained (Fig. 1). Afterwards, normal cells grow over the radioactive ones. Using L-coniferin, prepared from L-glucose and radioactive coniferyl alcohol, no radioactive lignin is obtained[16]. When radioactive phenylalanine is introduced, it is transformed into radioactive coniferin[17], which can be isolated from the plant after one or two days. After a further few days, we find once again that the radioactivity is fixed in that zone of cells which is in the process of lignification at the time of the experiment. It is astonishing how quickly the transformation of the phenylalanine into coniferyl alcohol, and the conversion of the latter to its glucoside, take place. Thus, coniferyl alcohol is derived from an acid of the C_6C_3 group, precursors of which are shikimic and prephenic acids.

We have, therefore, at our disposal ordinary natural lignin isolated from wood, lignin produced in the tree in the presence of radioactive coniferin, and lignin similarly formed in the presence of phenylalanine. Both coniferin and phenylalanine can be labelled at different carbon atoms.

But there is also a way of preparing lignin *in vitro*. If air is bubbled through a very dilute aqueous solution (less than 0·5 per cent) of coniferyl alcohol in the presence of mushroom laccase, at 20° C. and *p*H 5·5–6·5, a light grey-brown precipitate is formed. Depending on the enzyme concentration, the time required may be minutes or hours. The precipitate accounts for 80–90 per cent of the coniferyl alcohol used. From it some substances of low molecular weight can be extracted by butanol at ordinary temperatures, and the residue is biosynthetic lignin produced by enzymatic dehydrogenation of coniferyl alcohol. Cambium laccase can be used instead of mushroom

laccase[19], and the biosynthesis can also be effected by means of horse-radish peroxidase and very dilute hydrogen peroxide solution[19]. Radioactive coniferyl alcohol labelled at different carbon atoms can also be used for these *in vitro* experiments.

Our array of lignin preparations suitable for examination is therefore increased by these biosynthetic products. Extensive comparison, both physical and chemical, of the best biosynthetic and natural lignin preparations leads to the conclusion that they are identical in their properties[3,17,20,21]. Analogous degradation products from both were either radioactive or not, depending on the site of the labelled atoms in the monomeric tracer. The molecular weight in both cases was of the order of 10,000. Details may be seen in publications of the Heidelberg school.

The middle part of Fig. 2 represents a cross-section through the cambium of spruce wood; the cambium cells can be recognized by the nuclei which have been included. On the inner side of the cambium on the right, the delicate, newly formed cells are converted gradually into mature wood, while on the left they form bark. When the cell walls have become quite thick, wood formation is ended. The lower part of Fig. 2 shows a section which has been stained with phloroglucinol and hydrochloric acid. The lignin is coloured a deep red, and is indicated by dark portions on the drawing. During the vegetative period the tissues of the cambium and adjacent cells contain large amounts of the glucoside coniferin. Laccase and, to a smaller extent, peroxidase, are also present in the cell sap[19]. But these enzymes cannot attack coniferin; they can only build up lignin from coniferyl alcohol. This led to the conclusion that a β-glucosidase must also be present in the tissue, but we were unable to extract it. We therefore used a dilute solution of the synthetic glucoside, indican, which can be split by glucosidase into glucose and indoxyl. Indoxyl on exposure to the air is oxidized to indigo. (M. W. Beijerinck[23] used a somewhat similar method with the sap of *Isatis tinctoria* to indicate the location of emulsin in almond sections.) In our case the blue colour of indigo develops rapidly between the fresh cells and the mature wood (upper part of Fig. 2)[22]. Here are the very cells in which lignification is taking place. The prediction of the presence of a β-glucosidase and its direct observation are a strong argument in favour of our theory of the biogenetic pathway of formation of wood. Coniferin is produced from C_6C_3 acids *en route* from the point of introduction to that of lignification. It diffuses into the newly formed cells, where it is hydrolysed to glucose and coniferyl alcohol, and the latter, coming into contact with laccase and peroxidase, is transformed into lignin. The cell dies as soon as the hollow spaces of the cell wall are filled up with lignin.

To recapitulate: coniferin, which originates from a C_6C_3 acid, is an intermediary substance which is converted by the action of a β-glucosidase into coniferyl alcohol, and this hydroxy-cinnamic alcohol is then transformed by laccase and peroxidase into lignin. Natural lignin and lignin formed *in vitro* are identical. It is thus proved conclusively that spruce lignin is a C_6C_3 substance of the guaiacyl type, but the details of its constitution remain just as obscure as ever.

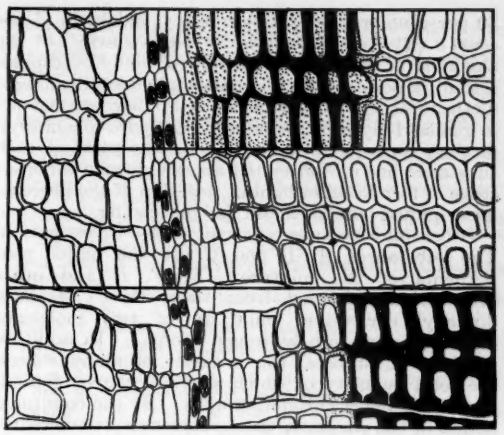
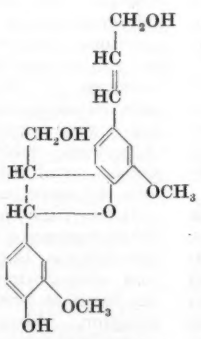


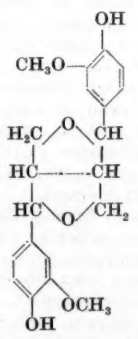
Fig. 2. Cross-section (schematic) through spruce cambium. Middle part unaltered, bottom part treated with phloroglucinol hydrochloride, top part treated with indican

However, there is one more way of approaching this question. As is mentioned above, the crude biosynthetic lignin contains substances of relatively low molecular weight. If lignin formation is interrupted at a certain stage, namely, when all the coniferyl alcohol has been used up and only very little lignin has been formed, a large amount of these intermediates of low molecular weight is obtained. The intermediates of the *in vitro* preparation form the vital key to the *in vivo* processes of lignin synthesis. So far, the following intermediates have been isolated[24–27]:

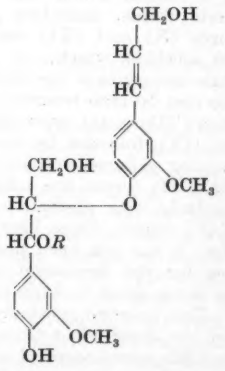
(*A*) dehydro-diconiferyl alcohol (V); approximate amount 20 per cent of the original amount of coniferyl alcohol; (*B*) DL-pinoresinol (VI); about 20 per cent; (*C*) guaiacylglycerol-β-coniferyl ether (VII); about



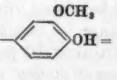
(V) Dehydro-diconiferyl alcohol (substance *A*)



(VI) Pinoresinol (substance *B*)



(VII) R = H: guaiacylglycerol-β-coniferyl ether (substance *C*)



(VIII) R = CH_2—CH=CH— guaiacylglycerol-diconiferyl diether (substance *H*)

(XIII) R = CH_3

(XIV) R = $C_{13}H_{11}O_{10}$

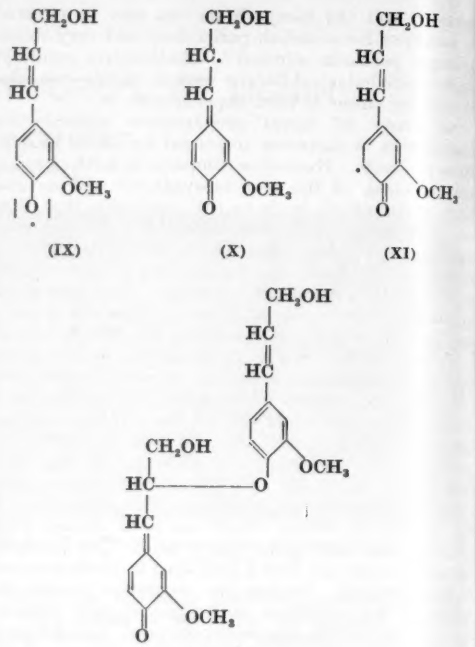
30 per cent or more; (*D*) coniferyl aldehyde (1 per cent); (*E*) the aldehyde of V (4 per cent); (*F*) the aldehyde of VII (3 per cent); (*G*) dehydro-dipinoresinol (4 per cent); (*H*) guaiacylglycerol-diconiferyl diether (VIII); 15 per cent. A further product (*J*) is guaiacyl-glycerol-β-coniferyl-γ-dehydro-diconiferyl-diether, corresponding to (VIII). The yield of each substance has its individual maximum at a different stage of formation of lignin. Addition of the percentages quoted above would be misleading.

Other intermediates are still under investigation. The substances *A*, *B* and *C* are the major constituents of the mixture; *E*, *F*, *G* and other products are derived from these three; *G* is a diphenyl derivative, built up of two molecules of (VI), minus two hydrogen atoms. In addition to these substances, the presence of many others, the total quantity of which is small, can be demonstrated by paper chromatography. By the continued action of the enzymes, these substances are dehydrogenated further and condense with each other or with coniferyl alcohol to form the lignin polymolecule[30]. A subsidiary amount of linking may be caused by a simple addition without loss of hydrogen. This is the case with substances *H*, formula (VIII), and *J*. There is evidence that such a condensation occurs through the addition of the primary carbinol group of coniferyl alcohol to a quinone methide group (formula XII, see below).

The above formulæ contain all the elements of structure which are necessary to explain the chemical behaviour of lignin: (VII) contains a phenyl carbinol group, (VI) and (VIII) contain aliphatic benzyl alkyl ethers, and (V) is a benzyl aryl ether. These groups are involved in the sulphite and sulphate pulping processes. Cinnamic aldehyde groups, which are responsible for the colour reactions of lignin, are also present. *Iso*hemipinic acid is obtained from (V) and is radioactive when the β-carbon atom of the coniferyl alcohol side-chain is labelled with carbon-14[17]. It is noteworthy that all these intermediates as well as lignin itself are optically inactive.

It is not difficult to explain the origin of all of these substances. Enzymatic attack on coniferyl alcohol causes the removal of a phenolic hydrogen atom, leaving a radical which can be written in various mesomeric forms, including (IX), (X) and (XI). Structures (X) and (XI) can combine to form a quinone methide which, by rearrangement, yields (V), while two units of the limiting structure (X) can combine and become rearranged to pinoresinol (VI). Similarly, (VII) could arise from the combination of (X) and (IX), followed by the addition of water to the resulting quinone methide (XII). With coniferyl alcohol, (XII) forms the labile substance (VIII)[27], and similarly with methanol or cane sugar, (XIII) and (XIV) result; these have been isolated.

So far, it has not been possible to furnish direct evidence for the formation of the radicals; their dilution is too great and life-time probably much too short. The quinone methides have been observed recently by direct ultra-violet measurements[28], and their half-life is estimated at about 60 min. under the conditions of measurement. Well-founded chemical evidence for the intermediate formation of (XII) has also been obtained. If the enzymatic dehydrogenation of coniferyl alcohol is carried out in water containing 30 per cent methanol, a new substance appears among the regular ones[28]. It is the γ-methyl ether (XIII) of (VII), and is formed by addition of methanol to the quinone methide (XII), thus providing direct



proof of the transitory existence of (XII). *J* is an addition product of (XII) and *A*. Even cane sugar can be added to (XII) in the same way, the product being (XIV)[28]. This demonstrates the manner in which lignin is combined with the carbohydrates of the cell wall.

The theory of lignin constitution developed above explains why the C_6C_3 units in conifer lignin, although originating from a single substance, namely, coniferyl alcohol, occur in such a vast number of different forms. Another remarkable fact is that lignin and also the intermediates, despite their many asymmetrical atoms, are all optically inactive. This is also explained by the intermediate formation and mesomerism of the radicals, which are optically inactive. The action of the enzymes is restricted to dehydrogenation of phenolic groups. All subsequent reactions, such as the formation of quinone methides by the interaction of the radicals, the addition reactions of the quinone methides and possibly polymerization reactions, proceed automatically without further regulation by enzymes. Even a solution of copper sulphate in water transforms—albeit slowly—coniferyl alcohol in the presence of oxygen into the same intermediates, and finally into lignin.

It has been mentioned above that the enzymatic dehydrogenation of coniferyl alcohol does not yield biosynthetic lignin quantitatively. The reason is that, even during the course of its formation *in vitro*, the lignin is partially degraded by further dehydrogenation. When a fine suspension of either natural or artificial lignin in water is subjected to the dehydrogenating activity of the enzymes[19] laccase or peroxidase, it undergoes the same degradation. This process is closely related to humification and destruction of phenols in the soil. Lignin can therefore be built up and degraded by the same enzymes. In the organized system in the plant, only formation occurs, because the action of the enzymes ceases when the

tissue has been filled up with lignin. But when the dehydrogenases approach from external sources, as in the case of attack by wood-rotting fungi, the degradation process sets in. Hence it is understandable why mushrooms and wood-rotting fungi contain laccase and peroxidase — preferentially in the mycelium and stipe—although they do not produce lignin. They require the laccase for the degradation of lignin or other phenolic substrates of the soil or wood.

Lignin of deciduous wood is built up of a dehydrogenated mixture of coniferyl and sinapinic alcohols (I and III). Sinapinic alcohol, which occurs in trees as its glucoside syringin (IV), has never been found as the exclusive building unit for a natural lignin. The explanation is given by the following experiment. When laccase or peroxidase acts on sinapinic alcohol, 80–90 per cent syringa-resinol (5 : 5′-dimethoxy derivative of pinoresinol, VI) is formed[19,29]. The residual 10–20 per cent consists of the same degradation products as are obtained when pure syringa-resinol is exposed to dehydrogenase[19]. Obviously the intermediates containing phenyl ether bonds corresponding to (V) and (VI) cannot be formed owing to steric hindrance. Following enzymatic degradation of syringa-resinol, 3 : 5-dimethoxy-*p*-quinone has been obtained[19].

The question is therefore answered as to whether lignin has an intelligible structural principle, and it is also explained why its architecture is more intricate than that of any other polymolecule[30]. So far, the principles involved in the formation of lignin have not been applied to the technical synthesis of other materials.

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INFLUENCE OF THE ANTERIOR PITUITARY ON THE AGEING PROCESS IN THE OVARY

By Dr. E. C. JONES and Prof. P. L. KROHN*

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TWO alternative ways of defining and measuring the rate of ageing are open to us. The first is an actuarial analysis of the increasing vulnerability of an ageing population. The second is based on the individual, and attempts a causal analysis of the ageing process at a chosen level of biological organization. For this purpose a particularly suitable method is to form heterochronic chimeras, in which tissues of one age are transplanted into the environment of another age. It is obviously desirable to choose a system for study which shows clear-cut signs of deterioration with age, but the visible changes that can be expressed quantitatively vary widely from one system to another. The central nervous system is one which could possibly be studied, and Burns[1], making use of Brody's[2] figures, has calculated that 100,000 neurones are irretrievably lost from the human cerebral cortex every day. The glomeruli in the kidney probably constitute another field of study. But neither appears to provide as advantageous a situation as does the female reproductive system, where the changes in overall reproductive capacity are very easily measured and can be correlated with a gradual loss of oocytes from the ovaries.

It has been universally accepted that the ovaries finally lose all the oocytes which they once contained. But since the middle of the nineteenth century there has been vigorous controversy about whether neoformation during adult life can add to the original complement or whether the female has to make the best she can of a limited number of oocytes with which she is endowed in early life. The problem has been exhaustively reviewed from time to time as opinion has oscillated in favour of one theory or the other. The most notable recent reviews[3,4] have been based on a series of quantitative observations and planned experiments which decisively tip the balance in favour of the endowment theory. These data clearly show that the decline in the total number of oocytes begins early in life and continues regularly with increasing age. They also show that no experimental situation has been contrived in which any part of the ovary can be convincingly demonstrated to give rise to fresh oocytes.

* Nuffield Senior Gerontological Research Fellow.

Fig. 1. Ovary from 346-day-old *CBA* strain mouse hypophysectomized 264 days earlier. (× 60)

Like the neurones, therefore, the female germ cells appear to be unable to reproduce themselves by mitotic division and are irreplaceable. It is because an understanding of this limiting factor would have such relevance to the study of the pattern of fertility that an analysis of the processes underlying the disappearance of oocytes has been undertaken.

Removal of oocytes at ovulation, and the atresia of other Graafian follicles which, for reasons that are not yet clear, fail to ovulate, can readily account for some of the loss. The decline in numbers, however, is too great and begins too early in life (immediately after birth in mice and rats), when there are no large follicles, for this factor to do more than provide a partial explanation. It has long been apparent that the major part of the loss must be due to some different process that affects the small follicles.

In addition to controlling the loss of oocytes at ovulation, it is possible that the pituitary may have a further influence on the ageing process in the ovary. The limited purpose of the present experiments was, therefore, first—to compare the rates of loss of oocytes in normal and hypophysectomized mice ; and second—having established that hypophysectomized animals lose their oocytes more slowly, to show by transplantation methods that the oocytes in ovaries, whose rate of ageing has been retarded by hypophysectomy, remain fully viable, even when their chronological age is greater than the age at which reproduction has ordinarily ceased. The labour involved in counting, and the limitations imposed by immunological considerations virtually restrict one's choice of animal in experiments of this kind to the mouse. All the experiments reported here have been carried out on mice of the *CBA* strain, whose exceptionally rapid normal loss of oocytes makes them particularly convenient to use. Further studies (to be reported elsewhere) have shown that the influence of hypophysectomy on the numbers of oocytes also extends to other strains (*A* and *R*III and hybrid crosses) in which we have not yet, however, carried out transplantation experiments.

Methods and Results

The mice were hypophysectomized at an average age of 39 days by Thomas's[5] method. Only those the sellæ turcicæ of which were shown by serial sections at the end of the experiment to be free of pituitary remnants have been included in the results. The technique adopted for counting the oocytes was the same as that used by Mandl and Zuckerman[4] in rats.

The results of the counts in 52 normal *CBA* mice, ranging in age from 1 to 437 days, can be expressed by the equation :

$$\log y \text{ (total number of oocytes)} = 4{\cdot}049 - 0{\cdot}00711\,x \quad \text{(days)}$$

and for 16 hypophysectomized *CBA* mice aged from 44 to 346 days at autopsy :

$$\log y = 3{\cdot}735 - 0{\cdot}00247\,x$$

The two regression coefficients are significantly different from each other ($P < 0{\cdot}001$). They imply that normal *CBA* ovaries lose about 30 per cent of their population of oocytes every 20 days and hypophysectomized animals only about 10 per cent. For example, the ovaries of a 320-day old normal *CBA* mouse would be expected to contain about 70 oocytes whereas the ovaries of a hypophysectomized mouse of the same age would contain about 880 oocytes—more than 12 times as many. The histological differences between the two types of ovary are exemplified in Figs. 1 and 2. The ovary of the hypophysectomized mouse (Fig. 1) is much smaller and contains neither large follicles nor corpora lutea. But there are far more small oocytes in it, and the usual signs associated with ageing in the ovary (for example, ceroid, pigment and anovular follicles) are much reduced.

There are no precise histological criteria which permit one to distinguish categorically between normal and atretic small follicles. Nevertheless, an attempt was made to separate the two categories when the original counts were being made. The ratio of normal to atretic small follicles was found to be 74 : 26 in hypophysectomized *CBA* mice and 49 : 51

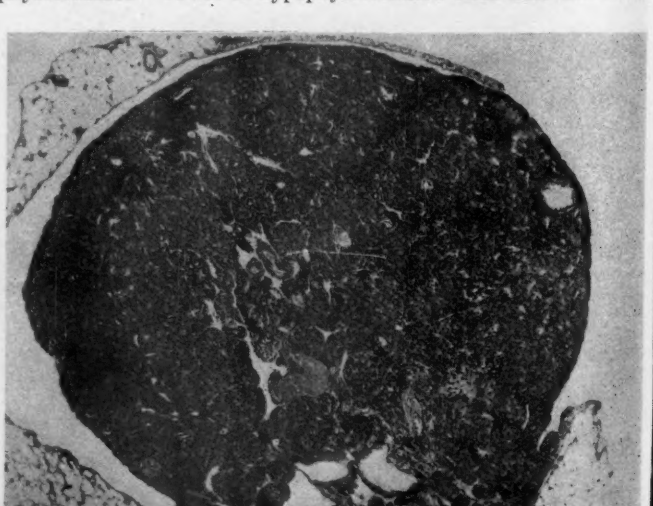

Fig. 2. Ovary from normal 360-day-old *CBA* strain mouse. (× 60)

in normal mice. Afterwards, five observers who were not told that the sample contained ovaries from hypophysectomized animals were asked to estimate the ratio of normal to atretic follicles in a smaller sample. They evidently varied in the subjective criteria which were used to assess the normality of an oocyte, since the proportion thought to be normal differed, but all agreed that the proportion was greater in those ovaries which had come from hypophysectomized animals.

The reproductive capacity of the ovaries of the hypophysectomized mice was tested by transplanting them orthotopically into young and old mice of the same strain, using a surgical technique which will be described elsewhere.

The first host mouse was a 456-day old *CBA* female which had given birth to 8 litters, the last 2 months earlier. Her ovaries, which were removed immediately before the unilateral orthotopic transplantation, contained only anovular follicles and remnants of corpora lutea. The hypophysectomized donor of the transplanted ovary was 311 days old and had been hypophysectomized for 273 days. The number of oocytes in the transplanted ovary was estimated from the regression equation at 460.

The host had previously shown irregular periods of vaginal cornification; her cycles remained infrequent and irregular after the grafting operation. She was paired with a male of proved fertility but never showed any signs of having mated.

The ovarian pad of fat, containing the graft and Fallopian tube, was removed 72 days later for histological examination. Serial sections showed that the operation had been technically successful, in that there were no obvious barriers to the free passage of ova from the graft to the Fallopian tube and uterus. The graft had enlarged and, among the total of 270 follicles which remained, contained 7 follicles with antra. There were, however, no corpora lutea nor signs of luteinization and the nuclei of stromal cells retained the cartwheel appearance, typical of a deficiency of luteinizing hormone, which is found with increasing frequency as animals get older[7].

Three young spayed virgin *CBA* females were given single orthotopic ovarian transplants. The first was 62 days old and received the second ovary from the hypophysectomized mouse which had provided the graft for the old mouse referred to above. Evidence that the graft was producing œstrogen soon appeared, the vaginal smear being fully cornified within a week. An interval of pseudo-pregnancy followed mating, and was succeeded by a pregnancy which resulted in the birth of three normal young. A second litter of two died, but a third litter of six was entirely healthy. The grafted ovary was 437 days old when the third litter was born, an age which is well past the average (352 days) at which reproduction ordinarily ceases in the *CBA* strain of mice.

The other two young females (92 days old) received single transplants (estimated to contain 290 oocytes each) from a 392-day old mouse hypophysectomized 354 days earlier. Vaginal cornification returned 4 and 5 days after the operation. One mouse has given birth to two litters, each containing two young, the second litter being born when the grafted ovary was 498 days old. The other mouse has mated repeatedly and has shown a succession of pseudo-pregnancies without becoming pregnant. It is assumed that adhesions have developed between the surface of the graft and the ovarian capsule which prevent the eggs from reaching the tube. This unfortunate complication of the operation is fairly common.

Discussion

The results show that after hypophysectomy oocytes are lost much more slowly from the ovary and that the proportion of small follicles showing the histological signs of atresia decreases. The ageing process, as it affects the ovary of the mouse, is therefore retarded when the pituitary is removed.

Earlier studies of the effect of hypophysectomy on the number of oocytes in the ovary purported to show that the number actually increased after hypophysectomy in rats[8,9]. The experiments were not, however, intended to show any relationship between pituitary secretions and the destruction of oocytes by atresia, but to prove that the pituitary ordinarily prevents the ovary from engaging in the neoformation of oocytes at the rate it would choose if freed from restraint. However, Ingram's[10] much more satisfactorily controlled experiments failed to confirm the observations on which this conclusion was based. Instead they clearly demonstrated that the number never exceeded the normal pre-operative control figures, and indeed, continued to decline after hypophysectomy. Although Ingram's hypophysectomized rats did lose their oocytes more slowly than the normal animals, he was unable to show that the difference between the two regression coefficients relating age and numbers of oocytes was statistically significant.

Our results can best be explained by assuming that hypophysectomy removes an unidentified atresia-promoting factor in addition to stopping the loss from ovulation. An alternative but more cumbersome explanation would be that some extra-pituitary factor is no longer able to act, the pituitary hormones normally playing the sort of permissive part which is sometimes attributed to adrenal cortical hormones in other endocrinological situations. It seems unlikely that the factor is any one of the gonadotrophins since the rapid loss of small follicles by atresia in the normal animal precedes any evidence that gonadotrophins are being released by the pituitary. It is tempting to choose somatotrophin from among the recognized hormones of the anterior pituitary, on the basis of its known influence on cellular growth and differentiation. At present there is, however, no experimental justification for such a choice.

But the population of oocytes does not remain intact even after the removal of the atresia-promoting factor by hypophysectomy. On the contrary, the negative slope of the regression line differs significantly from zero, and oocytes continue to be lost, even if more slowly than normal. It must therefore be assumed that yet another unknown process, which is independent of the pituitary, promotes the loss of oocytes. Ageing of the ovary, as measured by the overall loss of oocytes, therefore depends on the combined effects of at least three separate factors—one concerned with the maturation of follicles for ovulation, a second unidentified pituitary principle and a more general influence whose effect persists in the absence of the pituitary. As mentioned earlier, the cell populations of several of the other organs in the body are subjected to a process of attrition with the passage of time. Analysis revealed that this process, as it affects the ovary, has a complex structure, but no one knows how far the behaviour of the ovary is unique, or how far it represents a pattern which also applies to these other organs. Only

further experiments can decide whether, for example, the decline with age in the number of cortical neurones is retarded by hypophysectomy and whether some loss of cells persists even in the absence of the pituitary.

The transplantation experiments reveal, in addition, that the oocytes and follicles which remain after long-term hypophysectomy are entirely viable and do not necessarily deteriorate simply with the passage of time. They respond normally to the appropriate gonadotrophic stimuli; the follicles ovulate and if the ova are fertilized they are capable of developing into normal young. Equally, the corpora lutea which develop from old granulosa cells are able to maintain a normal pregnancy.

The behaviour of grafts of ovaries from a hypophysectomized mouse depended entirely on the nature of the environment in which they were placed. In an old host the ovary failed to do more than show that its small follicles could grow to Graafian size, whereas complete reproductive capacity was demonstrated in the young host. These observations reinforce earlier suggestions that, although the pituitary continues to produce gonadotrophins, it no longer does so in the proportions which are necessary for reproduction. In particular, it suggests the possibility that the production or the release of ovulating and luteinizing hormones is impaired.

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OBITUARIES

Mr. Frank Twyman, F.R.S.

The scientific world is the poorer for the passing of Mr. Frank Twyman on March 6. He was born in Canterbury in 1876, and attended Simon Langton's School in that city. He went to the Finsbury Technical College and later as Siemens Scholar to the Central Technical College, London. He then obtained a post as cable tester in the Fowler Wiring Cables Co., but he found the work irksome, especially night shifts, and went to Otto Hilger, as he characteristically put it, to "have a rest". (Later he described the work as "a most complicated way of earning a living".) Otto Hilger had succeeded his brother Adam in charge of the firm of Adam Hilger; he died in 1902 and the management passed to Twyman, although only twenty-six years old at the time. Two years later the firm was incorporated with Twyman as managing director, a post which he held until 1946. In 1948 the firm amalgamated with E. R. Watts and Son, and he served as a director until 1952, after which he became technical adviser.

Twyman was a man of tireless energy; until 1910 he designed all the new instruments completely, making all detailed drawings and testing and adjusting the instruments himself. He made many innovations in the production and testing of high-quality components, and was always vigilant in seeking new methods. He believed in sharing his knowledge with others, and for this reason published his book, "Prism and Lens Making". His developments in interferometers for testing optics alone would entitle him to a high place in innovation in industry. Twyman had a deep knowledge of glass-making and an original outlook which benefited makers of high-quality optical glass. He helped to start the Optical Materials Committee, which was of great value during the First World War.

Twyman's outstanding achievement was in the field of spectrochemical analysis. Although the possibility of the use of spectroscopy for chemical analysis, particularly of metals, had been known since the middle of the past century, it had not found favour. He became convinced of its importance, and in 1904 produced the constant-deviation spectrometer with the wave-lengths of the lines displayed on a helical drum. This was a success, but not with analysts. The range was restricted to the visible region, whereas the lines of most interest for metal analysis lie in the ultra-violet. Therefore, he designed the fixed medium quartz spectrograph, which shows a wide range of wave-lengths on a photographic plate, and also a large quartz spectrograph of greater dispersion. These appeared in 1910, but with one exception (the American Brass Co.) he failed to persuade analysts of their usefulness, although ready sales were made for academic purposes. Two events broke the deadlock: the American Brass Co. published the results of nine years successful routine-control of brass production, and two firms, one in Germany and one in the United States, made equivalent instruments and inspired the publication of investigations made by means of them.

He was also busy with work in other regions of the spectrum. He made vacuum ultra-violet spectrographs, the first going to Bohr's laboratory in Copenhagen. Against the advice of Paschen, who affirmed that the infra-red region would never be of industrial interest, he made an infra-red instrument, the first of several. X-ray spectrometers were made for Laby and others, and instruments for the investigation of crystal structures by X-rays.

Twyman was elected a Fellow of the Royal Society in 1924. The John Price Weatherill Medal of the Franklin Institute of Washington was presented to him in 1927, the Duddell Medal of the Physical Society in 1927 and the Gold Medal of the Society of Applied Spectroscopists of the U.S.A. in 1956.

He was interested in general matters also, bringing an original mind to bear upon economics and education. He published a small book entitled "Some New Proposals for the Amelioration of the Balance of Payments Position", and another called "Apprenticeship for a Skilled Trade". He was interested in music, gardening, literature and the theatre, and had a wide knowledge of old music-hall songs. For many years his lunch consisted of a glass of milk, which he drank to the accompaniment of his violin—he used to explain that he was "trying to become a third-class amateur". Those who worked with him knew that his apparent ingenuousness hid a clear

appraisal of the situation; when a junior brought him a problem, he would say, "and what do *you* recommend?" It is no wonder that he was much loved and respected by his staff. A. C. MENZIES

Prof. Thomas Thomson

WITH the recent death of Prof. T. Thomson we saw the passing of a person who, with singular devotion, had spent his whole life in the service of one educational institution.

When Thomson, a Londoner, entered the University College of North Wales, Bangor, in the first decade of this century, he was somewhat older than the average run of students at the time, and after reading for honours in chemistry, and taking a first, he continued to read forestry under Prof. Fraser Story, then head of that Department.

During the First World War, Story was called to London, and Thomson worked in Wales on problems of woodland felling and timber supplies. This gave him an admirable introduction to the Welsh countryside and to the mind of the countryman, a most valuable asset early in a professional career.

In 1919, the Forestry Commission was set up. Story stayed on in London as its education officer and Thomson returned to Bangor to take charge of the Forestry Department, first as an independent lecturer and latterly, before his retirement, eleven years or so ago, as professor. This was a period of expansion and adaptation in the subject, to meet the new and expanding economy of the new State forest policy in Britain, together with the continuance of training of forest officers for work in the Dominions and overseas.

It was also the period of transfer of activities from the Old Buildings in the Penrhyn Arms Hotel to the Memorial Buildings in Deiniol Road in the mid-twenties. It also witnessed the ever-expanding provision of field courses of training, extramural to the Department at Bangor. Before the War there had been established at Penisa'r Glyn near Chirk in the Ceiriog Valley, Denbighshire, an experimental forestry unit, in the layout of which Thomson was the prime mover. Now, after the War, there followed comprehensive and extensive practical forestry courses in the field in the French State forests, with their long established and tested traditions. This was eventually extended to embrace collateral courses in the newer State forests of Britain, as these developed and matured.

As an academic discipline the subject suffered severely at this time from the serious lack of authoritative standard text-books in English. This Prof. Thomson set himself to remedy, and in due course appeared his English translation of the classic text of "The Foundations of Silviculture" from the German of Busgen and Munch. This was followed by Thomson's own "Outline of Forestry", less ambitious in scope, but withal a very worthy students' manual.

During the Second World War, and the second severe onset on the native timber resources of Britain in one short generation, Thomson gave admirable service, outside his Department, in operating the State scheme of grants for new planting and replanting of timber trees. His thorough and extensive knowledge of conditions in the field in Wales, based on his initial experience during the First World War and extended since, and his natural endowments in the field of human relationship made him a first-rate agent in working this policy—indeed a very successful ambassador among estate and woodland owners.

He had retired from the chair before the Department made its second move to the present site at the corner of Deiniol Road and Glanrafon Hill, but it gladdened his heart to see, so firmly established and so full of promise to the future, a pulsating and virile school that he had done so much to bring into being and to support in its infancy.

Prof. Thomson served his College well in the field of internal administration—a field of service he rather delighted in—as curator of the College grounds, in Board of Faculty, in Senate and as Dean. He was never forward in the rough and tumble of argument and advocacy in the early stages of discussion of any issue, but invariably he came in towards the end with words of wisdom and sagacity. He was a very sound dialectician, a loyal colleague and a sturdy pillar of the Old Students' Association of the College.

Prof. Thomson was twice married, first to Miss Gwen Phillips, eldest daughter of the late Reginald W. Phillips, first professor of botany in the College, and secondly to Miss Connie Izard, a member of the College Music Trio, who survives him.

R. ALUN ROBERTS

Mr. Cyril Barnard

MR. CYRIL CUTHBERT BARNARD, librarian of the London School of Hygiene and Tropical Medicine, and author of the Barnard system of classification for medical libraries, was killed in a road accident on March 6. Son of Harry Barnard, an artist specializing in the design of Wedgwood ware, he was born at St. Margaret's, Middlesex, on July 23, 1894, and was educated at the Stationers' Company School and at the London School of Economics. In 1914, he became a library assistant at the Royal Society of Medicine and in 1918 assistant librarian at the Wellcome Historical Medical Library. During 1921–28 he served as librarian of the Tropical Diseases Library, and in 1929 took charge of the library of the London School of Hygiene and Tropical Medicine in its new building in Keppel Street.

Mr. Barnard graduated B.A. (London) with honours in 1922, and two years later obtained the diploma of the School of Librarianship. In 1931, he was awarded the honours diploma of the Library Association for a thesis entitled "A Classification for Medical Libraries", which was published in 1936. A second enlarged edition, "A Classification for Medical and Veterinary Libraries", came out in 1955. Mr. Barnard was elected a Fellow of the Library Association in 1925, served on the Council of the Association and as secretary of its University and Research Section, and in 1947 became chairman of the newly created Medical Section. In 1953, he was joint honorary secretary of the First International Congress on Medical Librarianship held in London. In 1952, he was invited by the World Health Organization to reclassify its library at Geneva according to his system. At his own library, which his expert knowledge and his devotion made one of the finest of its kind in the world, he introduced special courses of instruction in the use of the library for students.

A member of the Society of Friends, linguist, historian, and a friendly, cheerful, approachable, and quietly firm personality, Mr. Barnard will be greatly missed in medical library circles. W. R. BETT

NEWS and VIEWS

Anti-Locust Research Centre: Dr. B. P. Uvarov, C.M.G., F.R.S.

Dr. Boris Petrovitch Uvarov, who recently retired from the directorship of the Anti-Locust Research Centre, London, was born in Uralsk in 1888. At the end of the First World War he was a lecturer in zoology and entomology at the Georgian State University, Tiflis. He had already had a distinguished career in Russian entomology following his graduation at the University of St. Petersburg. It was while working as director of the Stavropol Bureau of Entomology on the control of locusts in 1912 that he recognized the conspecificity of two 'species' of locusts as the solitary and swarming phases of *Locusta migratoria*, a discovery which was to revolutionize work in this field.

Uvarov was also keenly interested in Acridid taxonomy, so that he was able to accept the invitation extended to him in 1920 by Dr. G. A. K. Marshall, director of the Imperial Bureau of Entomology, to work in London. As assistant entomologist and later as senior entomologist he worked at that Institute on the identification of Orthoptera and Hemiptera at the British Museum (Natural History). During this time he published numerous important papers on the taxonomy and biogeography of the Acrididae which brought him much acclaim and he rapidly became the world authority on this family. But Uvarov was more than a taxonomist, and such works as "Locusts and Grasshoppers" (1928), "Insect Nutrition and Metabolism" (1928) and "Insects and Climate" (1931) placed him in the forefront of entomological workers.

Meanwhile, the control of locusts was becoming more and more important and he began to devote most of his time to studying this problem. Towards the end of the Second World War the danger of famine in the Middle East and in East Africa became acute, owing to the desperate shortage of shipping. When, therefore, the Colonial Office set up the Anti-Locust Research Centre in 1945, Uvarov was appointed director. Under his inspired leadership and as a result of very hard work involving extensive travel, the Centre grew from strength to strength and is now generally recognized as the most important institute of its kind in the world. Its great reputation and international influence may safely be attributed to Dr. B. P. Uvarov. He was appointed C.M.G. in 1943, became a Fellow of the Royal Society in 1950 and is now president of the Royal Entomological Society, London. He holds various foreign decorations, including the Chevalier, Légion d'Honneur and Commandeur de l'Ordre Royal de Lion of Belgium.

Dr. T. H. C. Taylor

T. H. C. Taylor, who has been appointed to succeed Dr. Uvarov as director of the Anti-Locust Research Centre, graduated at Reading University College. In 1925 he went out to Fiji as government entomologist to assist Dr. J. D. Tothill in his investigation of the problem of the coconut moth (*Levuana iridescens*), which had been causing extensive damage to the coconut plantations. He travelled widely in Melanesia and Indonesia in search of parasites, and as a result of this work the moth was brought completely under control. The results were published by Tothill, Taylor and Paine in 1930 in a massive report on this classic example of successful biological control. In 1935 he was transferred to Uganda, where he specialized on cotton pests. In 1944 he joined the staff of the Commonwealth Institute of Entomology, becoming assistant director in succession to Dr. W. J. Hall, who had been promoted to director. In 1953 Dr. Taylor joined the Anti-Locust Research Centre as deputy director. Since then he has made many tours of inspection of locust stations in Arabia and Africa.

National Agricultural Advisory Service

Sir Robert Rae, director of the National Agricultural Advisory Service, who is to retire on July 31, was formerly professor of agriculture in the University of Reading (see *Nature*, 153, 400; 1944). In 1944 he was appointed British agricultural attaché in Washington and agricultural adviser to the United Kingdom High Commissioner in Ottawa. In 1945 he became provincial advisory officer in the National Agricultural Advisory Service, and was promoted to under-secretary in 1947. He was appointed director of the National Agricultural Advisory Service in 1948.

Sir Robert Rae is to be succeeded as director by Mr. J. A. McMillan, at present senior education and advisory officer. After being wounded in the First World War, Mr. McMillan graduated at the University of Edinburgh in 1919. He afterwards became lecturer in agriculture in the University of Leeds, and agricultural organizer to the Lincoln (Lindsey) and Cambridgeshire County Councils. He was seconded during the Second World War to the Cambridgeshire War Agricultural Executive Committee. On appointment to the National Agricultural Advisory Service in 1946 he became provincial director, Eastern Province. He was promoted to senior education and advisory officer in 1948.

Mr. W. E. Jones, at present director of the National Agricultural Advisory Service in Wales, who will succeed Mr. McMillan, graduated with honours in agriculture at the University College of Wales, Aberystwyth. In 1946 he was appointed to the National Agricultural Advisory Service as a grassland husbandry advisory officer, and in 1950 became county advisory officer. He was appointed deputy director for Wales in 1954, and in 1957 he became director of the Service in Wales.

Mr. H. E. Evans, at present deputy director of National Agricultural Advisory Service in Wales, who is to succeed Mr. Jones, graduated with honours in agriculture at the University College of North Wales in 1934. He then spent six years with the Agricultural Economics Department at Aberystwyth. He was associated with the War Agricultural Executive Committees in England from 1940 until he joined the National Agricultural Advisory Service when it was formed in October 1946. Since then Mr. Evans has had continuous experience of farming in Wales, having served as county advisory officer in both Radnor and Carmarthen and more recently as deputy director of the National Agricultural Advisory Service for Wales.

Institute of Oceanography, Dalhousie: Prof. Ronald Hayes

THE National Research Council of Canada has awarded an annual grant of 90,000 dollars to Dalhousie University, Halifax, Nova Scotia, for the establishment of an Institute of Oceanography. All branches of marine science will come under investigation, and opportunities for work at sea will be provided by the Royal Canadian Navy, the Fisheries Research Board of Canada and other agencies. Prof. F. R. Hayes, who has been appointed director of the Institute, took his doctorate under the late Prof. James Johnstone in the Department of Oceanography, University of Liverpool. As a post-doctoral fellow of the Rockefeller Foundation he worked at the Prussian Marine Station at Heligoland and the University of Kiel. He is at present chairman of the Division of Biological Sciences at Dalhousie University, and a member of the Fisheries Research Board and National Research Council of Canada.

Pressing problems of the Second World War brought a marked impetus to oceanography in Canada. Co-operation then established has continued under a Joint Committee on Oceanography with representatives of the Royal Canadian Navy, the Fisheries Research Board and the National Research Council, as well as Defence Research Board and the Hydrographic Service. Demands for expansion in the various fields brought to notice the lack of adequate provision for the training of recruits and for research unconnected with immediate economic aims. The Committee appealed to the National Research Council, and Dalhousie University proposed a programme of work, for which the present grant makes provision.

Staff appointments at Dalhousie will provide for research and teaching at graduate level in marine branches of biology, chemistry, geology, and physics. The oceanographic programme at Halifax is being expanded considerably. In 1958, the 177-ft. research vessel, *A. T. Cameron*, was brought into service. Built by the Fisheries Research Board, she has five laboratories, will accommodate nine scientists and carries a crew of 25. The Fisheries Research Board Atlantic oceanographic group, previously stationed at St. Andrews, New Brunswick, is moving to Halifax. The Naval Research Establishment has available a corvette, C.N.A.V. *Sackville*, and an algerine, C.N.A.V. *New Liskard*. It is anticipated that a second algerine will be assigned to research work. The Canadian hydrographic service has recently announced plans for a 4,200-ton research ship scheduled for completion in 1961. The vessel will carry a crew of 85 and scientific staff of 15–20. She will have ten laboratories and will carry two helicopters. The double hull will be reinforced for navigation in Arctic waters, and she will be fitted for all types of oceanographic research, including core-sampling of the bottom.

On the Pacific coast of Canada the University of British Columbia has operated an Institute of Oceanography for the past decade. A similar grant from the National Research Council will permit a very considerable expansion of the programme there also.

Appointments to National Research Council of Canada

THE following new members of the National Research Council of Canada have recently been appointed: Dr. F. R. Hayes, head of the department of zoology at Dalhousie University, Halifax; Dr. A. D. Misener, head of the department of physics, University of Western Ontario; Dr. J. Unrau, head of the department of plant science at the University of Alberta, Edmonton. The vacancies were created by the retirement of Dr. J. H. L. Johnstone, head of the department of physics and dean of graduate studies, Dalhousie University, and by the deaths of Dr. R. S. Jane, president of Shawinigan Chemicals Limited, and Dr. R. B. Miller, professor of zoology, University of Alberta.

Ciba Fellowships, 1959–60

THE Advisory Panel of the Ciba Fellowship Trust has awarded the following Fellowships for the academic year 1959–1960: Dr. M. H. Richmond (University of Cambridge and the Medical Research Council) to study at the University of Copenhagen (*microbiology*); Mr. V. P. Arya (University of Banaras and of London) to study at the Polytechnic, Zurich (chemistry of natural products); Mr. J. F. Counsell (University of Bristol) to study at the University of Göttingen (physics); Mr. G. L. Duncan (University of Aberdeen) to study at the University of Louvain (polymer chemistry); Mr. K. Jones (University of Sheffield) to study at the University of Heidelberg (organic chemistry); Mr. B. L. Mordike (University of Birmingham and of Cambridge) to study at the Max Planck Institute, Stuttgart (physical metallurgy).

Imperial College of Science and Technology: Fellowships

THE governing body of the Imperial College of Science and Technology, London, has made the following elections to Fellowship of the College: Sir Lewis Casson, former student of the City and Guilds College; Sir Graham Cunningham, member of the governing body of the Imperial College; Prof. A. Holmes, professor emeritus of geology in the University of Edinburgh, a former student of the Royal College of Science; Sir Christopher Ingold, professor of chemistry, University College, London, former student and member of staff of the Imperial College; Dr. L. B. Pfeil, director of the Development and Research Department of the Mond Nickel Company, former student of the Royal School of Mines; Mr. R. McKinnon Wood, member of the governing body of the Imperial College.

Television Society Premiums

THE Television Society has awarded the following premiums, for outstanding papers read before the London meetings during 1957–58: *The Wireless World* Premium to Mr. B. Overton (Mullard Research Laboratories) for his paper on "Transistors in Television Receivers"; the E.M.I. Premium to Dr. R. Theile (Institut für Rundfunktechnik) for his paper on "Recent Investigations into the Operation of Image Orthicon Camera Tubes"; the *Electronic Engineering* Premium to Dr. J. C. Parr (formerly of Kelvin and Hughes, Ltd.) for his paper on "Some Aspects of Waveguide Technique"; the Pye Premium to Mr. L. J. Griffen (Kolster Brandes, Ltd.) for his paper on "Dressing Television: Cabinet Design"; the Mervyn Premium to Mr. K. H. Smith, (Siemens Edison Swan, Ltd.) for his paper on "Performance of Television Receiver Turret Tuners"; the Mullard Premium to Mr. J. Polonsky (Compagnie Générale de Telegraphie sans Fils) for his paper on "A French Portable Television Camera".

Committee on Chemicals, Oil and Gas from Coal

In the House of Commons on April 16, Mr. J. C. George asked the Paymaster-General what further action the Government proposed to take to investigate possible methods of converting coal into chemicals, gas and oil. Sir Ian Horobin, in a written answer, stated that the Minister of Power has decided, after consultation with Sir Alexander Fleck, chairman of the Scientific Advisory Council, to set up a committee with the following terms of reference: "To review the work that has been done in recent years on the development of processes in which coal is the basic raw material and which will produce marketable products of a chemical or a gaseous or liquid hydrocarbon type; and to make recommendations as to the direction of further research and development work on any such processes which appear to the committee to hold promise of industrial application and as to the type of organization or organizations best suited to carry out such work".

The members of the committee will be: *Chairman*, Mr. A. H. Wilson (managing director in charge of research and development, Courtaulds, Ltd.); *Secretary*, Mr. W. R. G. Bell (Ministry of Power); Mr. M. A. L. Banks (director, B.P. Trading, Ltd.); Mr. Henry Benson (Cooper Brothers and Co.); Mr. H. E. Collins (National Coal Board); Capt. (E) W. Gregson (chairman, Fuel Efficiency Advisory Committee); Dr. R. Holroyd (director, Imperial Chemical Industries, Ltd.); Mr. W. K. Hutchison (chairman, South Eastern Gas Board); Dr. M. A. Matthews (Shell International Chemical Co., Ltd.); Mr. B. E. A. Vigers (Laporte Industries, Ltd.). The committee will be assisted in its work by two assessors: Mr. A. W. Clarke (director, Warren Spring Laboratory of the Department of Scientific and Industrial Research); and Mr. D. A. B. Llewellyn (Ministry of Power).

Manufacture of Polypropylene in Britain

An agreement has been signed whereby Imperial Chemical Industries, Ltd., acquires a licence under the Montecatini and Montecatini/Ziegler U.K. patents covering the production and use of a new polypropylene plastic, originally discovered by Prof. Natta. A new plant to manufacture this plastic ('Propathene') is being constructed at the Wilton Works in North Yorkshire. It is expected to bring the total capacity of Imperial Chemical Industries for polyolefines for 'Alkathene' and 'Propathene' to more than 100,000 tons a year. 'Propathene' is being manufactured at a pilot plant, and arrangements have been made to augment this pilot plant production so that the material will be available commercially from June 1. 'Propathene' is particularly suitable for moulding uses in view of its high rigidity and resistance to temperature. It is also expected to find extensive use in film form and as a general extrusion material.

First Hungarian Atomic Reactor

Hungary's first experimental atomic reactor, which began working on March 25, is a small reactor, of 2 MW. capacity, situated in the Buda Mountains above Budapest. The installation is fully automatic. The Soviet Union supplied plans and a large part of the installation, five Hungarian institutes drew up the final designs and thirty Hungarian establishments took part in the construction of the reactor. It is primarily for research work, and particularly for the training of nuclear technicians. Neutrons obtained from the reactor will be used for experimental examination of the structure of the atomic nucleus, and isotopes will be supplied to industrial, medical and scientific establishments in Hungary. Previously, radioactive isotopes required for therapeutic purposes have been obtained abroad, and short-lived isotopes required for biological experiments could not be obtained at all. The reactor will solve both these problems. Experience gained from the new reactor will be used in the future planning and construction of atomic power stations in Hungary.

The National Engineering Laboratory

The Council for Scientific and Industrial Research has announced that in future the Mechanical Engineering Research Laboratory at East Kilbride, near Glasgow, will be known as the National Engineering Laboratory. This is considered desirable in order to emphasize the national character of the Laboratory, which is part of the Department of Scientific and Industrial Research organization and is financed from public funds: it does not imply any change in the field covered by the Laboratory. A Steering Committee has been set up to look after the programme of the Laboratory, the object of which is to extend knowledge of mechanical engineering science so as to provide industry with the information it requires for the solution of its own particular problems. In order therefore that its work may be focused on practical objectives of real value to industry (which may involve basic or applied research or, in suitable cases, development work), it has been decided that the planning of the Laboratory's activities and the selection of research projects can best be under the control of a small body with a measure of executive authority. The Committee's terms of reference are: (1) to be responsible to the Research Council for the selection of projects and for the allocation of effort to them, within the financial resources allocated to the Laboratory and such other limits as may from time to time be imposed by the Council; (2) to report annually to Council, submitting a research programme for the following year. The Committee, which will be a full committee of the Research Council, is empowered to set up specialist sub-committees to provide technical advice on particular fields of work.

The new Steering Committee will be under the chairmanship of Vice-Admiral Sir Frank Mason, (director of Metal Industries, Ltd., and of H. W. Kearns and Co., Ltd.), who is a member of the Research Council and chairman of the outgoing Mechanical Engineering Research Board. The members of the Committee will be Prof. O. A. Saunders (Imperial College of Science and Technology, London), Norman Elce (director of Metropolitan-Vickers Electrical Co., Ltd.), Dr. D. G. Sopwith (director of the Mechanical Engineering Research Laboratory), Dr. C. M. Cawley (director of Stations and Grants Divisions of the Department of Scientific and Industrial Research). In order to assist the Director as the Laboratory expands and to improve and extend the links between the Laboratory and industry, two deputy directors have been appointed. They are Dr. S. P. Hutton and Mr. F. D. Penny. Dr. Hutton, who is at present head of the Fluid Mechanics Division of the Mechanical Engineering Research Laboratory, will have special responsibilities for fostering closer relationships with industry. Mr. Penny, who is chief development engineer at the Fuel Research Station, will be responsible for the management of the research programme.

Technical Assistance Programmes

At the request of the International Co-operation Administration, the U.S. National Academy of Sciences—National Research Council has undertaken a study of the ways in which science and technology can most effectively be used by the Administration in its assistance programmes for Africa south of the Sahara and north of the Union of South Africa. Leaders in scientific and technological research and African studies have been asked to assist in the evaluation of opportunities for the utilization of research in the fields of public health, medicine, agriculture, natural resources, engineering and education. A final report will be submitted by June 1959. Dr. J. George Harrar, director for agriculture at the Rockefeller Foundation, has been appointed executive director of the study.

Laboratory Equipment in American Schools

Teachers of science in American colleges, universities and non-profit-making organizations have been invited by the National Science Foundation to submit proposals for the construction of better laboratory equipment for use in schools. Under the terms of a new, experimental programme, the Foundation will consider proposals for the design and construction of improved laboratory equipment and for the development of new instructional materials for lecture demonstrations, laboratory and field work for courses at elementary and secondary school, and undergraduate levels in mathematics, astronomy, earth sciences, physical and biological sciences, and engineering. Support will not be provided for the purchase of equipment for renovating school and college laboratories, or for commercial production of equipment or materials.

Nigeria Science Association

As a result of the rapidly increasing volume of scientific work in Nigeria, many scientists have for some time felt the need for a Nigeria Science Association. In October 1957 a meeting was called at the University College, Ibadan, to discuss the matter. After a further meeting in January 1958 it was decided that a Science Association of Nigeria should be formed and should hold its inaugural meeting in December 1958. A steering committee was elected, with Prof. J. Grayson, professor of physiology at University College, Ibadan, as chairman, and Dr. Brian Hopkins, lecturer in agricultural botany at University College, Ibadan, as secretary. The inaugural meeting of the new Association was held at University College, Ibadan, during December 15–19, 1958. At a brief but impressive opening ceremony, which was well attended, the Association was inaugurated by the Hon. Victor Mukete, Federal Minister for Research and Information. The opening ceremony was followed by a conference lasting four days. The first three days were devoted to papers and discussions of general interest and to business meetings, and the last day to specialist sections. Four specialist sections were organized: medical sciences, physical sciences, botany and zoology. At the business meeting, Dr. Eni Njoku, Department of Botany, University College, Ibadan, was elected first president, and Dr. Hopkins secretary. It is hoped that the next conference will be held in Zaria in December 1959.

Frederick Soddy Trust

The first report of the Frederick Soddy Trust (pp. 16. From the Frederick Soddy Trust, 9 The Drive, Hove 3, 1958) includes a full record of grants made up to September 1958 as well as a list of rejected applications, with the view of indicating the principles used in selection. Emphasis is placed on team-work rather than individual work, and on actual study and research in the field. Some details are given of the survey of crofting in South Uist and Benbecula made in 1957; of the proposed study of Corby and of a survey of the parish of Ardington, Berkshire, proposed by the University of Reading; and also of an expedition to the Pyrénées in which a study is to be made of peasant agriculture and its response to a mountain environment; an attempt is to be made to evaluate the extent of possible population movement in the area.

Smoke Control

Under the title "Smoke is Your Enemy" (pp. 8, 1959) the Ministry of Housing and Local Government and the Central Office of Information have prepared a short pamphlet for issue to householders in areas which local councils are proposing to make smoke control areas. The pamphlet explains simply what smoke control will mean to the household, the grants available to the householder and the advantages which smoke control will bring.

Exhibition of German Measuring Equipment

An exhibition of precision measuring equipment, made by Carl Zeiss (Oberkochen) and by M. Hensoldt and Soehne (Wetzlar) and mainly of interest to those working in the fields of engineering metrology and metallurgy, was held during March 9–14, in the showrooms of Degenhardt and Co., Ltd., at 6 Cavendish Square, London. Although the equipment displayed consisted mainly of new versions of well-known measuring and observing instruments, their new look made careful scrutiny necessary in some instances before identification with one or other of their famous prototypes could be made. In general, no claims were advanced for enhanced accuracy of performance over previous attainments, but outstanding improvements have been made in such matters as ease of manipulation of controls and adjustments. These new features considerably reduce the strain of continuous observing and contribute to the readier achievement by the user of the full potentialities of accuracy available in well-designed instruments. Ease of control was especially noticeable in the microscopes, of which the 'Ultraphot' camera microscope and the smaller Zeiss photomicroscope are typical examples; in addition, each is provided with a built-in automatic camera, and exposure time is regulated photoelectrically. The 'Technoscope', a binocular low-power microscope giving five different magnifications for the same working distance, has applications in the laboratory, the engineering workshop and the operating theatre. The range of engineering metrology equipment included the universal, tool-makers', interference and light-section microscopes, optical dividing heads, rotary dividing tables, alignment telescopes and a number of smaller measuring aids and devices.

Organization for European Economic Co-operation Courses on Nuclear Science

The European Nuclear Energy Agency of the Organization for European Economic Co-operation has arranged with the Atomic Energy Research Establishment at Harwell and the Centre d'Études Nucléaires at Saclay (France) for two international

courses on nuclear energy. Designed for the teaching staffs of universities and higher technical colleges, each course will accept about fifty applicants, who will have the opportunity to study the latest advances in nuclear science and techniques, to do certain practical work, and to visit nuclear installations. The costs of these courses, both of which will run during July 20–31, are being borne by the European Nuclear Energy Agency, and participants will only be required to pay travel and living expenses.

The Night Sky in May

New moon occurs on May 7d. 20h. 11m. U.T. and full moon on May 22d. 12h. 56m. The following conjunctions with the Moon take place: May 11d. 14h., Venus 7° N.; May 13d. 03h., Mars 7° N.; May 22d. 06h., Jupiter 3° S.; May 24d. 21h., Saturn 4° S. In addition to these conjunctions with the Moon, Mars is in conjunction with Pollux on May 21d. 06h., Mars being 5·3° S., and Venus with Pollux on May 31d. 18h., Venus being 4·2° S. Mercury is too close to the Sun for observation. Venus is an evening star, visible in the south-west after sunset. Conditions are very favourable for observation. Venus sets at 23h. 00m., 23h. 20m. and 23h. 25m. on May 1, 15 and 31, respectively; its stellar magnitude is −3·7. Its distance decreases during the month from 104 to 82 million miles and the visible portion of the disk decreases from 0·734 to 0·607. Mars is moving eastwards among the stars and is in Gemini during May. It sets at 0h. 40m., 0h. 10m. and 23h. 35m. at the beginning, middle and end of the month, respectively. Its stellar magnitude decreases from +1·6 to +1·9 during the month, its distance from the Earth increasing from 173 to 197 million miles. Jupiter rises at 21h. 00m., 19h. 55m. and 18h. 30m. on May 1, 15 and 31, respectively, setting at 5h. 35m., 4h. 35m. and 3h. 20m. It is in Libra. The stellar magnitude of Jupiter is −2·1; its distance from the Earth on May 15 is approximately 410 million miles. Saturn is in Sagittarius, rising at 0h. 00m., 23h. 05m. and 21h. 55m. at the beginning, middle and end of the month, respectively. Its stellar magnitude is +0·5 and its distance approximately 870 million miles. The only occultation of a star brighter than magnitude 6 is that of 115 Tau, on May 10d. 20h. 08·3m. (disappearance at Greenwich).

Announcements

Lord Rothschild is joining the Board of 'Shell' Research, Ltd., on May 1 as part-time adviser on research. He is an assistant director of research in the Department of Zoology, University of Cambridge; until recently, he was chairman of the Agricultural Research Council, a post he occupied for ten years.

Dr. Walter R. Beam, manager of microwave advanced development in the Electron Tube Division of the Radio Corporation of America, has been appointed head of the Department of Electrical Engineering at Rensselaer Polytechnic Institute, Troy, New York.

An English translation of the *Astronomical Journal of the Academy of Sciences, U.S.S.R.*, issued in six parts annually, is being published by the American Institute of Physics in co-operation with the National Science Foundation, under the title *Soviet Astronomy—AJ*. The annual subscription is 25 dollars in the United States, or 27 dollars elsewhere, with special rates for university libraries. Orders and inquiries should be addressed to Translation Journals—*AJ*, 335 East 45 Street, New York 17.

The Glasshouse Crops Research Institute, Worthing Road, Rustington, Littlehampton, Sussex, will hold an open day on June 10. Particulars can be obtained from the Secretary of the Institute.

A conference on the Electric Arc in Welding will be held at the University of Birmingham on June 25. Further information can be obtained from the Secretary, Department of Industrial Metallurgy, University of Birmingham, Edgbaston, Birmingham 15.

An exhibition to commemorate the centenary of the death of Isambard Kingdom Brunel will be held in the main entrance hall of the Engineering Laboratory, Trumpington Street, Cambridge, during May 18–June 19, from 9 a.m. to 5 p.m. on weekdays and 9 a.m. to 12 noon on Saturdays. The exhibition will consist mainly of drawings and photographs of Brunel's principal works.

An exhibition of photometric instruments and microscopes applicable to most industrial processes and almost every branch of medicine, science and research will be held during May 11–21 in the showrooms of Degenhardt and Co., Ltd., United Kingdom agents for Carl Zeiss, at 6 Cavendish Square, London, W.1. Throughout the exhibition four technical experts from the Zeiss works at Oberkochen will be in attendance to give advice on the applications of the instruments.

The Editorial Board of the *Journal of Embryology and Experimental Morphology* is sponsoring the fourth of its series of International Embryological Conferences at the Collège de France, Paris, during September 21–24. Details of its organization and scientific programme will be available on June 1 and can then be obtained from Prof. E. Wolff, Laboratoire d'Embryologie Experimentale, 49*bis* Avenue de la Belle Gabrielle, Nogent-sur-Marne, France, or from Dr. L. Brent, Department of Zoology, University College, Gower Street, London, W.C.1, England.

The 1959 conference of the Operational Research Society will be held at the Old Swan Hotel, Harrogate, during June 10–12. The fee is £15. The object of the conference is to provide an opportunity to explore and discuss possible directions for the future development of operational research, particularly in the fields of communications and decision making. Copies of the programme and application forms can be obtained from the Secretary, Operational Research Society, 64 Cannon Street, London, E.C.4.

The fourth International Conference on Ionization Phenomena in Gases will be held in Uppsala, Sweden, during August 17–21. The conference is being arranged by the University of Uppsala in collaboration with the Swedish National Committee for Physics. The main subjects will be: 1, fundamental processes in gas discharges; 2, high-frequency discharges; 3, glow discharges; 4, arc discharges; 5, spark discharges; 6, high-temperature plasmas and controlled fusion; 7, instabilities and oscillations in gas discharges; 8, new methods of measurements. Abstracts, not longer than 350 words, should be received by June 15. Further information can be obtained from the secretary general, Dr. Åke Nilsson, Institute of Physics, Uppsala.

THE COMMONWEALTH ADVISORY COMMITTEE ON DEFENCE SCIENCE

MEETING IN AUSTRALIA

THE Commonwealth Advisory Committee on Defence Science exists to develop the mutual interest of the countries of the British Commonwealth in defence science. Formal meetings are held at two- to three-year intervals in various member countries. Continuity is given to the work, between meetings, by a permanent Working Party located in London. The Working Party is made up of permanent representatives of member countries and is concerned with reviewing progress and planning for future meetings.

To date, five official meetings have been held in addition to a preliminary informal meeting which was held in 1946. Two of the formal meetings have been held in London, one in India, one in Canada and one recently in Australia.

Meetings are attended by senior officers from the Fighting Services and the Defence Scientific Services of the member countries, and it is an indication of the importance attached to the meetings that officers of the highest status are nominated as delegates.

Credit for the creation of the Commonwealth Advisory Committee on Defence Science rests largely with Sir Henry Tizard, who held a prominent position in defence science in the United Kingdom during the post-war period. One of the first acts of the meeting held recently in Australia was to send him a cable of greeting.

In proposing the preliminary or informal Conference which was held in 1946, the United Kingdom authorities suggested that the Conference might consider the distribution of defence research activities throughout the British Commonwealth so as to make the best possible use of the available and potential facilities and staff in each country. This has continued to be a goal for succeeding Conferences.

One of the decisions of the preliminary meeting in 1946 was that there should be close collaboration with the United States of America in the work of the Committee. Its constitution as a Commonwealth body precluded the United States from becoming a member; but a close association has been maintained, and at all meetings of the Committee American observers have been present by invitation and have made significant contributions to the discussions.

The fifth official meeting of the Committee was held in Australia during the period of October 7–28. It was attended by some sixty delegates, approximately forty of whom were from overseas. Countries represented were Australia, Canada, India, New Zealand, Pakistan, South Africa and the United Kingdom, and in addition, a party of observers from the United States of America attended.

On this occasion Ceylon, the Federation of Rhodesia and Nyasaland, Ghana and Malaya were unable to be represented but are being kept informed of the Conference discussions.

On this occasion the general chairman of the meeting was Sir Leslie Martin, defence scientific adviser and chairman of the Defence Research and Development Policy Committee in Australia. Delegations, which were comprised of senior representatives of the Fighting Services and the Defence Scientific Services of the member countries, were led by: Australia, Mr. W. A. S. Butement, chief scientist, Department of Supply; Canada, Dr. J. L. Keyston, vice-chairman, Defence Research Board; India, Brigadier Y. B. Gulati, director of weapons and equipment, Army Headquarters; New Zealand, Dr. C. D. Ellyett, Defence Science Advisers Committee; Pakistan, Dr. Mohammed Afaf, senior scientific officer, Defence Ministry; South Africa, Dr. S. Biesheuval, director, National Institute for Personnel Research; United Kingdom, Sir John Carroll, deputy controller, Research and Development, Admiralty; United States (observers), Mr. J. B. Macaulay, deputy assistant secretary, Research and Engineering, Department of Defense.

In planning the meeting, it was considered important that while full opportunity should be given for adequate Conference discussions, there should also be the opportunity for delegates to obtain a broad picture of defence science activities in Australia. This would also provide the occasion to see something of the geography and general development of the country.

To achieve these objectives, the itinerary provided for arrival of delegates in Sydney, the official opening in Canberra, the main business sessions to be held in Melbourne and Adelaide and for visits to be made to places of scientific service and general interest. This itinerary involved some 3,000 miles travel by air and, therefore, introduced problems of organization, but the results were such that the effort was considered well worth while.

The Conference was opened by the Prime Minister of Australia, the Hon. R. G. Menzies, in Canberra on October 7. In his address, Mr. Menzies directed attention to two points which were important in relation to the social implications of defence science. He commented that, because of the glamour attached to the subject at the present time, there was a tendency for the public to think of defence science as being concerned solely with new and revolutionary developments of a most complex character. While much of the work was, of course, of this nature, he as a layman was most impressed in studying the Conference agenda to find that there was still a great deal of research to be carried out on such everyday items as food, clothing and the weather.

The other point which Mr. Menzies commended to delegates was that defence science was being presented to the public in a way which caused them to feel that these very great efforts were being made solely for military purposes and were, therefore, very largely of a destructive character. He considered that this was far from the truth, and he was of the opinion that there were few defence scientific developments of recent years where the potential application for civil purposes was not of far greater importance than the military application.

The agenda was made up of some seventy technical papers in addition to the reports of the sub-committees on Service psychology and defence food research and the group on agricultural aspects of defence science. The papers were circulated in advance, and at the Conference they were introduced briefly by named delegates and then discussed by the whole meeting. The papers fell largely in the broad fields covered by the above groups and in the fields of career development, telecommunications, tropical warfare, weapons testing, naval research cold-weather operations, effects of high-level noise and the effects of nuclear weapons. However, in addition, there were many individual papers outside these groups.

The subject of tropical warfare was one of special interest to several of the countries represented, in that their territories embraced large areas of tropical terrain. Numerous papers were discussed, ranging from the effect of the environment on the soldier and his equipment, the need for more efficient vehicles and aircraft for use in tropical areas, mental stress under tropical conditions, navigation and communication in the jungle, clearance of vegetation, corrosion in the tropics, etc.

The weapons-testing theme covered the biggest group of papers discussed at the Conference. The subject was interpreted in its broadest sense, aeroplanes, for example, being treated as weapons. This theme was of great interest to Australia, where two of the major defence science activities, namely, the Woomera Missile Testing Range and the Maralinga Atomic Testing Range, are both concerned very largely with the testing of weapons.

In particular, the Woomera project was discussed in some detail. Numerous papers dealing with the equipment of the Range, various aspects of the trials work and the processing of the results were considered. The discussions were supplemented by a comprehensive visit to Woomera, when the facilities were examined in detail and missile trials were witnessed.

While at Woomera, delegates inspected the missile-tracking station which had been established as part of the International Geophysical Year programme.

The visit to Woomera was of particular interest to delegates. The basic conception of this project arose largely from the early discussions by the Commonwealth Advisory Committee on Defence Science, and it was a matter of considerable satisfaction to delegates to see the very tangible result that had been produced. Sir John Carroll, leader of the British delegation, in a public address, referred to Woomera as the outstanding example of defence science co-operation in the Free World.

Because of the importance attached to seeing at first hand something of the scientific work proceeding in Australia, several visits were arranged to scientific and appropriate Service establishments. Included in these were the Aeronautical Research Laboratories, the Defence Standards Laboratories, the Weapons Research Establishments at Salisbury and the testing range at Woomera, the Maralinga Atomic Testing Establishments and the Edinburgh and Woomera Royal Air Force Establishments.

Quite apart from these visits, it was felt that it would be of interest for delegates to be given opportunities of seeing something of the country and of work proceeding in other technical fields. For this purpose, a two-day visit was arranged to the Snowy Mountains Scheme in the south-eastern corner of Australia. This is the largest engineering undertaking in Australia, and can compare in magnitude with the largest undertakings in other parts of the world. Although primarily concerned with the conservation of water by means of river diversion and large-scale storage, it is also a major generator of electric power, having a potential capacity of some 2,000,000 kW.

Numerous opportunities were included in the programme to enable delegates to meet Australians in many walks of life. Delegates were honoured by being entertained to lunch by the Government of the Commonwealth and by being received by the Governors of Victoria and South Australia and by the Lord Mayor of Melbourne. In addition to such official functions, several pleasant informal functions including visits to places of interest and private entertainments were arranged—visits to outback sheep stations and to the Andamooka opal fields.

At the close of the Conference, delegates were unanimous that it was one of the most successful held to date. The tangible results are, of course, a series of recommendations arising from three weeks of intensive discussions. These recommendations are for the guidance of member countries; they will be submitted to the respective governments for ratification, when they will become a valuable guide to defence science policies. The other result of the Conference is the intangible one arising from the friendships established between delegates and the mutual appreciation of the special problems faced by member countries.

LESLIE MARTIN

AUTOMATIC REDUCTION OF GEOPHYSICAL DATA

OVER the past two decades there has been a rapid increase in the use of automatic photographic and electronic techniques for recording experimental results. As a result, the number of measurements made need no longer be restricted by fatigue in the experimenter, and sets of results containing millions of measurements are not uncommon. The rate at which results can be accumulated is now so great that the individuals responsible for setting up the equipment can no longer assimilate the information at the rate at which it is produced. This problem can be overcome to some extent by arranging the programme of analysis in such a way that the reduction of data can be dealt with by semi-skilled staff.

Nowadays even this solution is often inadequate. Not only is it difficult to find suitable staff, but also many of the investigations require the analysis of data on a scale so great as to be beyond the capabilities of a team of individuals. The problem is particularly acute in the field of geophysical research, where very large quantities of results are frequently produced in the course of field work of many kinds.

The automatic reduction of geophysical data was the subject of a Geophysical Discussion organized by the Royal Astronomical Society and held on January 23 under the chairmanship of Sir Edward Bullard. Although the discussion was not primarily concerned with the results of the International Geophysical Year, it was appropriate that it should have been arranged at a time when many individuals must be giving consideration to the study of the unprecedented wealth of new information obtained during the preceding eighteen months.

Sir Edward opened the discussion by pointing out that a single measurement made in each degree square of the Earth's surface would amount to a total of nearly 70,000 readings, and that facilities for handling large numbers of data are almost essential in many types of geophysical work. Even in mobile experiments it was most desirable to arrange, if at all possible, for the results to be recorded directly in digital form. This saved a great deal of time later, since it avoided the need for manual punching on tape or cards, and it frequently made it possible to record data at a rate which would not be possible by other methods.

Sir Edward described his recent voyage in *Discovery II*, in the course of which 90,000 magnetic readings were made near the Azores. The results were recorded directly on punched tape together with other essential information which would be needed during the reduction stage. These included the date, time, records of clock adjustments and range changes and also the ship's position and heading. The programme for the computer was written in such a way that the whole reduction process could be dealt with in one stage. As the principal objective of the work was the study of small irregularities in the Earth's field, it was arranged that the regular component of the field should be removed as part of the computer programme.

Mr. R. H. Merson (Royal Aircraft Establishment) described some investigations of the orbits of artificial Earth satellites. The original aim of the work was to predict the times of future transits in order to enable more accurate positional data to be recorded. Later, it was found possible to gain valuable geodetic information from the results. The approximate size and shape of the orbit are first determined on a desk calculator. This orbit is then adjusted by successive approximations, using a digital computer, to give a least-squares fit with all the available observational information.

The accuracy of the observations is such that it has been possible to derive new values for the coefficients J and D in Jeffreys's expression for the Earth's gravitational potential and to make deductions concerning atmospheric drag on satellites.

The prediction of future transits of Earth satellites requires the rapid processing of incoming data if the results are to be produced in time to be of use. The conditions under which weather forecasts are produced are similar and provide an obvious reason for the use of a digital computer. Mr. G. A. Corby (Meteorological Office) discussed preliminary tests on the preparation of numerical forecasts with the aid of a computer. Barometric pressure and wind-speed measurements are received on tape in a variety of codes at a rate of about 100,000 a day and, to save time, the incoming tapes are decoded in the computer before being used for further processing. The object is to construct a contour map, showing the height of a constant-pressure surface over Europe and the North Atlantic, which accurately represents the observational data. In the least-squares fitting process, it is necessary to minimize the differences between the observed and calculated values of both pressure and wind speed. An additional requirement is that in the determination of the contours for any area, maximum weight must be given to the data from the nearest weather stations. The speed and accuracy with which the forecasts can be produced by automatic methods are satisfactory even though the techniques are still in the development stage.

Mr. M. H. P. Bott (University of Durham) discussed the use of a digital computer for determining the extent of geological formations from surface gravity anomalies. The anomaly due to a model consisting of a series of vertical rectangular prisms of arbitrary depth has been calculated. In the study of a particular formation the procedure adopted is to define a model which leads to the same calculated anomaly as that observed in the area under investigation. Providing the density of the formation is known, the form of the lower surface can be determined uniquely by this method. The technique has been successfully used on the Triassic rocks in Dumfries.

Dr. D. C. Martin (Royal Society) outlined briefly the arrangements which had been made for the collection of data during the International Geophysical Year. Full sets of data would be held in World Data Centres and selected data would be published in Annals of the International Geophysical Year. Although no plans had been made in advance for the automatic processing of the whole of these data, it seemed likely that much of the information would eventually be dealt with by machine methods.

Mr. M. J. Tucker (National Institute of Oceanography) discussed various techniques used in the spectral analysis of geophysical time-series with particular reference to the study of sea waves. Although the analysis can be carried out by deriving the Fourier components of the wave motion, it is often more satisfactory to express the results in terms of the power spectrum or the autocorrelation function.

Proposals for the application of a digital computer to the forecasting of the future behaviour of the ionosphere were outlined by Mr. C. M. Minnis (Radio Research Station). Present intentions are to represent the characteristics of the $F2$-layer in terms of a family of linear functions of solar activity based on all the past observational data. This can be achieved to a satisfactory degree of accuracy if an ionospheric index is used to represent solar activity. The intercepts and slopes which define the linear relations would be stored in a digital computer and those needed to make a forecast for a specified location, season, time of day and level of solar activity would be selected and used in conjunction with other data, in the later stages of the calculations. It is hoped that by this means it will be possible to make forecasts of a more practically useful type than those made using standard manual methods.

In many types of geophysical investigation the use of an automatic digital computer is almost essential if accurate results are to be produced quickly, but it must not be assumed that this is always so. Three speakers referred to work which was being carried out successfully and economically by other methods.

Mr. A. Dennison (British Petroleum Company) described the echo method for the exploration of underground geological formations and the difficulties

associated with the usual 'single spot' method of recording the results. Variable-density and variable-area recording had also been tried and the resulting records, when suitably displayed, could give a striking visual picture of the density variations in the underlying structures without any of the computations normally associated with echo soundings.

The analysis of Earth tide records obtained using Milne–Shaw pendulums was explained by Mr. G. W. Lennon (Liverpool Observatory and Tidal Institute), who enumerated reasons why a digital computer would not be appropriate in this type of work. For example, the removal of spurious diurnal and semi-diurnal oscillations would be difficult to programme for a computer, quite apart from the impossibility of producing long records without discontinuities.

Finally, Mr. H. M. Iyer (National Institute of Oceanography) referred to the lack of interest in the study of microseisms. If properly analysed they could yield useful information, and Mr. Iyer described experimental equipment which had recently been developed for the automatic recording of several statistical characteristics of microseisms.

C. M. MINNIS

CHEMISTRY OF CONDENSED TANNINS

THE Plant Phenolics Group held a meeting on "The Chemistry of Condensed Tannins" at Queen Elizabeth College, University of London, on January 6 under the chairmanship of Prof. R. D. Haworth (University of Sheffield). More than sixty members and guests were present.

In the opening paper on "Acid-catalysed Polymerization of Phenolic Compounds", Dr. B. R. Brown (Oxford) briefly summarized past work on this subject and indicated a possible reaction mechanism. *p*-Methoxybenzyl alcohols condense with phenols preferentially in a position *para* to a hydroxyl group to yield diphenylmethanes. A suitably substituted flavan, for example 4′-methoxyflavan, undergoes this condensation via ring-fission. Condensation of 4′-methoxyflavan with 7-hydroxyflavan yields a compound containing two flavan units the structure of which is based upon the known *para*-orientation of these reactions.

The importance of flavan-3 : 4-diols in tannin chemistry has led to an investigation of flavan-4-ol, which condenses with phenols, and with 7-hydroxyflavan yields a flavan 'dimer' isolated as the crystalline acetate.

These condensations, like the acid-polymerization of catechin, require high acid concentrations and it is unlikely that they are involved in the conversion of catechins into tannins in plants. This work aims at establishing the structural details of this condensation and at producing 'polymers' of known structure which can be used for the trial of new degradative methods, which may also be valuable for the degradation of condensed tannins.

Dr. D. E. Hathway (British Leather Manufacturers' Research Association), in a paper entitled "Oxidative Polymerization Mechanisms for Catechins", first briefly reviewed the chemistry of the oxidation of *o*- and *p*-dihydroxy- and *vic*-trihydroxy-phenols, which he illustrated by reference to the synthesis of the mould metabolite atromentin, to the formation of purpurogallin and the synthetic melanins, and to the transformation of gallates into ellagates. Possible mechanisms for the oxidation of coniferyl alcohol by laccase, and their relevance to lignin and lignan formation were also discussed.

Studies on the oxidation of (+)-catechin in aqueous and non-aqueous media and on the autoxidation of flavans related to catechin confirm that autoxidation of catechin to a polymer (catechin–tannin) proceeds via the corresponding *o*-quinone followed by intermolecular C—C linkage. The evidence suggests that the most likely linkage is between the 8-carbon of one molecule and the 6′-carbon of the second. The isolation, albeit in small yield, of *m*-hemipinic acid by oxidative degradation of the methylated reduced polymer substantiates this supposition. Further confirmation for the presence of head-to-tail units in the polymer was obtained from a study of the autoxidation and the enzymic oxidation of mixed substrates which resemble the head and tail of (+)-catechin. Enzymic oxidation by mushroom, potato and tobacco polyphenoloxidases proceeds at a faster rate and lower temperature than the autoxidation of catechin, and gives a product precisely similar to the autoxidation polymer.

The heartwood of *Acacia catechu* and the leaves of *Uncaria gambir* contain considerable quantities of (−)-*epi*catechin and (+)-catechin, respectively. The isolation, therefore, of purified tanning fractions in good yield from these sources, with properties similar to the autoxidation and enzymic oxidation polymers of catechin, strongly suggests that these tannins are formed from *epi*catechin and catechin, respectively, by the above mechanism.

Studies on the formation of oak-bark tannin indicate that (+)-catechin, (+)-gallocatechin and leucodelphinidin, which are formed in the leaves, are translocated via the sieve-tube system of the phloem to the cambium, where preferential enzymic oxidation of the pyrogallol phenols occurs. The resulting tail-to-tail polymer is stored in the bark. The remaining tannin action of the bark can be attributed to the occurrence of complex leucoanthocyanins.

In a paper on "Oxidative Condensation in Tea Fermentation", Dr. E. A. H. Roberts (Indian Tea Association) said that, according to Hathway and Seakins, oxidation of (+)-catechin is followed by a condensation between the catechol-*o*-quinone group of one molecule and the phloroglucinol ring of another. If phloroglucinol is added to an enzyme–catechol system, different intermediate- and end-products are formed, indicating a condensation of *o*-benzoquinone with highly active phloroglucinol. Oxidation of pyrogallol to purpurogallin, however, is not affected by the presence of phloroglucinol, which illustrates the very high reactivity associated with the hydroxy-*o*-benzoquinone system. Enzymic oxidations of (−)-*epi*gallocatechin and its gallate are similarly unaffected by the addition of phloroglucinol, but the addition of pyrogallol to these systems yields derivatives of purpurogallin (flavanotropolones). These observations suggest that, following oxidation

of the gallocatechins, a condensation takes place between the pyrogallol groups of two separate molecules.

Gallic acid and pyrogallol will not condense to form purpurogallincarboxylic acid unless both substances are oxidized, and this suggests that in the gallocatechin series the condensation takes place between two oxidized molecules. This is supported by the results of a study of the conditions required for the formation of purpurogallin.

The oxidations of (−)-*epi*gallocatechin and its gallate are followed by a linkage of two molecules of the resultant *o*-quinones to form the *o*-quinone of a bisflavanol with the two pyrogallol groups joined by a C—C linkage. Further condensations of this type are excluded on steric grounds, and it is suggested that the primary condensation products undergo either oxidation or reduction. Reduction leads to the formation of one of three possible bisflavanols, whereas oxidation, if a galloyl group is present, could lead to the formation of one of two purpurogallin derivatives. The available evidence supports the view that the theaflavins of black tea have these structures.

Prof. K. Freudenberg (Heidelberg) said that the term leucoanthocyani(di)n was a misnomer for those naturally occuring substances which on treatment with hot mineral acid either under aerobic or anaerobic conditions yield anthocyanidins. He proposed that they be named either 'pre- or pro-anthocyanins'.

Dr. T. Swain (Low Temperature Research Station, Cambridge), in introducing his paper on "Methods for the Investigation of Complex Leucoanthocyanins", also referred to the confusion in the nomenclature of leucoanthocyanins. He suggested that leucoanthocyanins of known structure be called by their systematic name, whereas polymers and co-polymers of hydroxyflavans ('flavols') be known as 'flavolans'. Those flavolans which yielded anthocyanidins on treatment with hot mineral acid would be known as 'flavylogens'. Dr. Swain then described methods for separating polymeric leucoanthocyanins into size groups and for analysis of their end groups.

Mr. W. E. Hillis (Division of Forest Products, Commonwealth Scientific and Industrial Research Organization, Australia), in his paper on the formation of condensed tannins in plants (read by Dr. E. C. Bate-Smith), recalled the probable role of shikimic acid and related alicyclic acids in the biosynthesis of polyphenols. Shikimic acid is present in appreciable quantities in the actively proliferating cambium of eucalypts. A linear relation exists between the amounts of shikimic acid and the weights of enlarging leaves of young *Eucalyptus sieberiana* trees, but this relationship does not hold for the fully-grown leaf; later the amount of shikimic acid in mature leaves diminishes with age. The final stages of the biosynthesis of shikimic acid can occur in the dark, and therefore shikimic acid present elsewhere in the tree need not necessarily originate in the leaves. The distribution of shikimic acid in leaves differs from that of the soluble polyphenols.

Comparative studies of the polyphenols in the cambium, sapwood and heartwood of three *Eucalyptus* species revealed the same general pattern of polyphenols, but shikimic acid was absent from the heartwood and only traces occurred in the developing sapwood. Catechin was less in the heartwood, whereas ellagic and gallic acids increased. On the basis of these facts a possible scheme for the biosynthesis of the heartwood polyphenols was outlined.

Mr. Hillis recalled that he had previously suggested (Society of Leather Trades Chemists Symposium on Vegetable Tannins, 1956, p. 127) that as certain tannins contain appreciable amounts of polymeric leucoanthocyanins, monomeric forms, originating in the leaves, could be the precursors of these substances. Present information, however, favours the formation of heartwood leucoanthocyanins *in situ*. Adaptation of the leucoanthocyanin reection has enabled the distribution of leucoanthocyanins in plant tissues to be studied by means of coloured photomicrographs.

In dealing with flavans of the resorcinol series in relation to the chemistry of commercially important condensed tannins, Dr. D. G. Roux (Leather Industries Research Institute, Grahamstown, South Africa) said that an examination of previous work on wattle and quebrecho extracts indicated that no direct evidence had been provided for the occurrence of catechins or flavan-3 : 4-diols as basic units in the corresponding tannins. The stereochemistry of the recently isolated black wattle catechin (−)-3 : 7 : 3′ : 4′ : 5′-pentahydroxyflavan and (−)-7 : 3′ : 4′-trihydroxyflavan-3 : 4-diol of quebracho heartwood was discussed. The progress of condensation of these compounds in the wood was examined by anthocyanidin formation, by number average molecular weights and by tannin analyses on radial and vertical drillings. These methods afford possible biochemical evidence of the presence of leucoanthocyanidins as intact flavan-3 : 4-diol units in these condensed tannins. The mode of cross-linkage in these condensed tannins must allow for its labile nature.

The chairman encouraged a vigorous discussion at the end of each paper, and among those participating were Drs. White and King (Forestal Central Laboratories), Bate-Smith, Thompson and Whalley.

J. W. T. Seakins

BAMBOOS AND NUTMEGS

TWO important taxonomic studies have recently been published in *The Gardens' Bulletin* (Singapore, 16, 1958), namely, "Bamboos of Malaya" by R. E. Holttum, and "A Revision of the Malayan Myristicaceae" by J. Sinclair.

Holttum has pointed out that the classification of the bamboos, as of other Gramineae, is based on inflorescence, flower and fruit characters; and in so far as such work tends to be done in herbaria, remote from the tropical collecting grounds, much information that would be of great use to the field botanist fails to be included. In fact, what the field botanist mostly sees are bamboos not in flower. Hence the value of vegetative characters, in particular the culm-sheaths, in field observations. This, indeed, is the kind of information possessed by the Malays, by whom the many kinds of bamboo are put to manifold domestic and other uses. Accordingly, in the present very full and informative work, classifications based on both vegetative and reproductive characters are given,

together with many excellent line drawings. Botanists and foresters will note with interest an observation on the flowering of bamboos in Malaya. In the equable climate which prevails there, the simultaneous, gregarious flowering of single species in particular areas, sometimes at intervals of several years, which is a well-known phenomenon in India, apparently does not occur. On the contrary, the native Malayan species can continue to grow indefinitely, flower sporadically from time to time, or in some instances, flowering a little almost continuously; the gregarious dying-back after a heavy flowering, as in India, is at most only an occasional feature of the Malayan scene. Among others, an item of considerable interest is a close analysis and comparison of the inflorescences in bamboos and grasses.

In revising the Myristicaceae, Sinclair has pointed out that a regional flora of this group, as, indeed, of any other group, is likely to be unsatisfactory unless it is seen against the considerably wider background of the species present in the whole of the adjacent Malaysian region. Thus, many species formerly considered to be confined to Malaya are now known to occur in Sumatra and Borneo. The difficulties encountered in the systematic treatment of the group are discussed. In the present revision, 53 species, 5 varieties and 1 form are described, whereas, in Ridley's "Flora", the equivalent numbers were 45 species and 4 varieties. Six new species are described. The text is well illustrated with line drawings and with photographs showing bark characters.

AMERICAN ARACHNIDA

THE spider family Dictynidæ has only recently been separated from the allied Amaurobiidæ, and its North American species are here revised and described. It is a family of world-wide distribution, with more than thirty genera and three hundred species, predominantly found in the north temperate zone. A dozen are British.

The spiders themselves are small spinners of cribellate webs in a variety of situations, and are unusual in that many species are gregarious, living together in very large numbers. In some areas in the States their thick communal webs choke ornamental conifers and evergreens and so give the spiders an economic importance. Their general habits and mode of life are but little known, and of their courtship and mating even less has been described. As a whole they seem to belong to the spring and summer, at which season alone males are to be found. The females lay only a few eggs, enclosed in lenticular cocoons, which are hung in the webs.

A recent paper by Prof. R. V. Chamberlin and W. J. Gertsch* describes eight American genera and 159 species, 119 of which are placed in the genus Dictyna. All these are fully described with the close attention to detail that modern arachnology requires, and distribution maps are added for many of the commonest species. The paper ends with 47 plates, carrying more than 600 admirable drawings, the work of Miss Marjorie Statham, whose artistry has enriched so many recent publications from the American Museum of Natural History.

It is obvious that this is one of the most painstaking revisions of a family of spiders that has appeared in recent years, and the authors must be congratulated on their achievement. At the same time its true significance can be properly judged only if two other points are mentioned. The first is that the authors themselves describe the revision as "still in many ways a preliminary one", since very large collections of Dictynidæ are now held both in New York and Utah. Here is an indication of the amount of systematic work which even a single family of spiders may involve.

The second point is that this paper recalls others, comparable if not quite so large, lately published in the *Bulletin of the American Museum of Natural History*. Dr. Levi's revision of the genus Enoplognatha and Dr. Muma's survey of American Solpugida are conspicuous examples. These have been supplemented by the smaller but no less desirable publications in the lively series, now some two thousand in number, called *American Museum Novitates*. No fewer than twenty-six of these, all dealing with the systematic side of arachnology, have come into my laboratory during the past few years. Obviously, there is a vigorous and fertile school of arachnology in America, which is taking its share, and more, in the very considerable task of ordering the Arachnida of the world in a coherent, reliable and accurate system. THEODORE SAVORY

* *Bulletin of the American Museum of Natural History*. Vol. 116, Article 1: The Spider Family Dictynidae in America North of Mexico. By Prof. Ralph V. Chamberlin and Willis J. Gertsch. Pp. 1–152 + plates 1–47. (New York: American Museum of Natural History, 1958.) 3.50 dollars.

OXYGEN AS A PREREQUISITE TO THE ORIGIN OF THE METAZOA

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DURING recent years there has been a revival of interest in the problems of the origin of life. This has led to speculation and experimentation, mostly along biochemical and biophysical lines. Less attention has been focused on the problems of the next steps in organic evolution, namely, those attending the diversification of primitive living forms into the kingdoms of organisms recognized to-day.

One of the important questions concerns the sudden appearance of highly organized metazoans in the fossil record. To state this more specifically: Why are there no fossil records of animal life from earlier

periods than the Cambrian, the lower reaches of which are characterized by trilobites? These are far too complex to have been the first animals that appeared on Earth, yet we have no traces of primitive ancestors to them. In Pre-Cambrian rocks there have been found some things that may be fossil sponge spicules and worm burrows, but that is about all. Various theories have been advanced to explain the lack of Pre-Cambrian fossils[1], but none is really satisfactory. However, the general intimation is that a long period of animal evolution must have occurred prior to the appearance of the trilobites, even although no traces remain. Recently Axelrod[2] postulated that the late Pre-Cambrian fauna might have developed in the littoral and immediate shore areas of the seas; these areas are subject to rapid and drastic erosion, a circumstance which does not favour the fossilization and preservation of their inhabitants. Hence Pre-Cambrian fossils should be rare, but they ought to exist. Axelrod suggests that particular care be used in seeking Pre-Cambrian deposits that can be identified as having originated in shallow seas, and that careful examination be made for the fossil remains of microscopic animals.

I wish to suggest that the lack of faunal remains in Pre-Cambrian deposits indicates the non-existence of a metazoan fauna for the greatest part of that period. I agree with Cloud[3] that there was a rapid (tachytelic) diversification of metazoans in late Pre-Cambrian times, and with Axelrod that the early forms were probably minute. But I wish to argue that the rapid diversification and increase in numbers was brought about by one specific ecological change, namely, by the addition to the environment of a surplus of free oxygen. To support this point of view the following facts and considerations can be marshalled.

In Pre-Cambrian rocks, fossil algæ have been found which precipitated calcium carbonate about themselves in various manners. Of these, *Collenia* is the best-known genus. One supposed alga, *Corycium enigmaticum* Sederholm, is of Archæan age which, if the correlation cited by Rankama[4] is correct, makes the organism 900–1,200 million years old[5]. In addition to such named forms, sporadically occurring carbon deposits have been taken to indicate that life was present and abundant in some Pre-Cambrian times, although of what form or degree of development we do not know.

It is, I think, generally considered that the early atmosphere of the Earth was reducing. Molecular oxygen was foreign to the environment, having been used up in inorganic oxidations. Various chemical reactions have been proposed to explain the advent of oxygen in the atmosphere, but the activities of green plants seem to offer the best explanation of its presence.

The exciting advances made during the past couple of decades in the biochemistry of photosynthesis have demonstrated how chlorophyl molecules could have been built up from inorganic substances through organic substances such as glycine and succinic acid to protoporphyrins and finally complex porphyrin compounds[6]. Photosynthetic reactions could originate anærobically, with oxygen being produced through the photolysis of water.

I suggest the following steps as a possible sequence of events. The primitive forms of life were heterotrophes, absorbing as their nutrients organic substances dissolved in the seas in which they lived. These heterotrophes may have been of several origins and existed in various habitats. Their subsequent evolution would involve considerable 'experimentation' in efficiency of anærobic metabolism. The next step was the development of protochlorophylls by certain types of organisms which would allow the utilization of the energy of sunlight for the reduction of carbon dioxide and its fixation in the form of carbohydrates. The organisms with this ability had thus become autotrophic. If, as has been suggested by several authors, the dissolved organic substances of the seas were becoming depleted after millions of years of forage by heterotrophes, these autotrophes possessed a competitive advantage, namely, freedom from dependence on these substances. They spread rapidly throughout the seas of the Earth. With them went an increasing concentration of an excretory product, oxygen. Oxygen spread through the waters and, by diffusion, into the atmosphere above the waters.

The development of the autotrophes ensured the survival of some heterotrophes as predaceous or carrion-feeding forms. The specialization and spread of autotrophic forms took further millions of years as the process of photosynthesis was improved. This would be the period of development and deployment of algæ. During this time, supported by these plants, there also lived a nondescript assemblage of heterotrophes. The heterotrophes were probably extremely small, perhaps in the form of colloidal aggregates. Of these as yet there has been found no identifiable trace.

Not until the concentration of oxygen in the hydrosphere and atmosphere reached a level at which it became a constant part of the environment did it exert a significant effect on the evolution of the heterotrophes. That is to say, not until there was more than enough oxygen present to take care of the continuing demand of inorganic oxidations was there sufficient to enable organisms to utilize it metabolically. To reach this level of free oxygen required the passage of vast periods of time. Not until the late Pre-Cambrian was this condition reached, but once oxygen became a constant element in the environment, oxidative metabolism with its advantages rapidly became established. Both heterotrophes and autotrophes could partake of these advantages. A variety of primitive forms could respond to the altered environment by using the new, more efficient method of extracting energy from their food. The consequence of such metabolic improvement was the development of metazoan and metaphytic forms through tissue differentiation. It is interesting to note that the fossil record indicates that animals developed earlier into varieties of complex forms than did plants. This suggests that some environmental factor was of more immediate use to animals than to plants. Oxygen was just such a factor.

To summarize, I suggest that (1) the lack of Pre-Cambrian metazoan fossils can be explained primarily by the assumption that there were few metazoans to be fossilized; (2) the addition of free oxygen to the environment as a constant feature only some 600 million years ago initiated a tachytelic development of metazoans (Urey gives 800 million years ago as the latest time to which a reducing atmosphere could have persisted[7]); but the amount of free oxygen required to initiate oxidative metabolism as a prerequisite of life may well have been much more than that required to enter inorganic reactions, so one can picture a period of, say, 200 million years during

which there existed barely minimal conditions for partially oxidative life; (3) life spent 1,000 million years or more becoming organized anærobically in the pre-Cambrian seas. This stage ended about 600 million years ago when the concentration of free oxygen reached a level sufficiently high to make it a constant feature of the environment; this enabled some organisms to undertake oxidative metabolism. The subsequent abundance and diversity of metazoan life formed the fossil record as we know it.

I am indebted to Dr. Hans Kalmus for many stimulating and clarifying comments made during the time these ideas were being put in order.

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DEDUCTION OF IONOSPHERIC ELECTRON CONTENT FROM THE FARADAY FADING OF SIGNALS FROM ARTIFICIAL EARTH SATELLITES

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ONE of the most marked characteristics of the radio signals received from the Earth satellites has been the complex fading of the received signal. It is here shown that from a study of the fading it is possible to deduce the electron content for the sample of the ionosphere between the satellite and the receiving station for a large section of the observed trajectory. This gives a sample across the sky, and can give information about the region above the F_2 maximum which is unexplorable by the methods of ionospheric sounding.

These fadings fall into two classes; first, those due to a change in the energy flux at the receiving aerial; and secondly, those accompanying a rotation of the plane of polarization of this energy. These two classes may be distinguished by the comparison of the signals received simultaneously on three orthogonal dipoles. For the first class, the fades will be in phase for all aerials, whereas for the second class there will be relative phase shifts depending upon the direction of arrival of the signal with respect to the dipole axes.

For signals received on a dipole from a satellite moving on a trajectory above an ionized layer, there will be a fading due to the variation in the Faraday rotation of the plane of polarization and also a fading due to the spin of the satellite. It can be shown that the variation in Faraday rotation can arise because of changes in: (*a*) electron content between satellite and receiver; (*b*) intensity of the Earth's magnetic field; and (*c*) angle between this field and the path of the ray in the ionized region.

Hence the rate of fading observed for transits in or above the *F*-region will be that due to the change in Faraday rotation which may be unmodified, increased or decreased by twice the spin-frequency of the satellite. During many transits a sudden change of twice the spin-frequency was observed in the rate of fading when the reception geometry changed; such a change appears in Fig. 1.

In order to evaluate the electron content corresponding to any part of the trajectory, it is necessary to know the absolute number of rotations of the plane of polarization due to the Faraday effect, as the signal travels from satellite to receiver. In the case of a ray passing normal to the Earth's magnetic field in the ionosphere, the Faraday effect will be very small and to a first approximation may be equated to zero.

This condition will occur at two points on any transit of the satellite, but they may be beyond the observer's radio horizon. However, for observers in Britain and trajectories such as those of the Russian Earth satellites, one of these points may be above the observer's radio horizon when the satellite is at a low angle of elevation north of the observer. The angle of elevation of this point increases as the observer moves farther south towards the magnetic equator. When one of these points is near or above the radio horizon, it is possible to count the number of Faraday rotations of the received signal as the

Table 1

Transit date, time, G.M.T.	n_s (Faraday rotation)		Ionogram		Geomagnetic latitude	$(h_s - h_m F_2)$ km.
	Farnborough	Cambridge	n_s	n_T		
October 18, 1957 03.30		8·2 5·5	7·0 4·8	4·6 3·9	Slough (54°) Inverness (60°)	40 10
October 19, 1957 03.30	6·4 2·8	6·8 2·9	6·9 3·5	4·0 3·5	Slough Inverness	50 0
November 7, 1957 14.00	31·8 15·5	28·2 16·5	47·5 16·5	14·8 9·4	Slough Inverness	240 160

n_T is the number of electrons ($\times 10^{-13}$) in a cm.^{2} vertical column up to the height of F_2 maximum as deduced from ionograms; n_s is the number of electrons ($\times 10^{-13}$) in a cm.^{2} vertical column up to the height of the satellite.

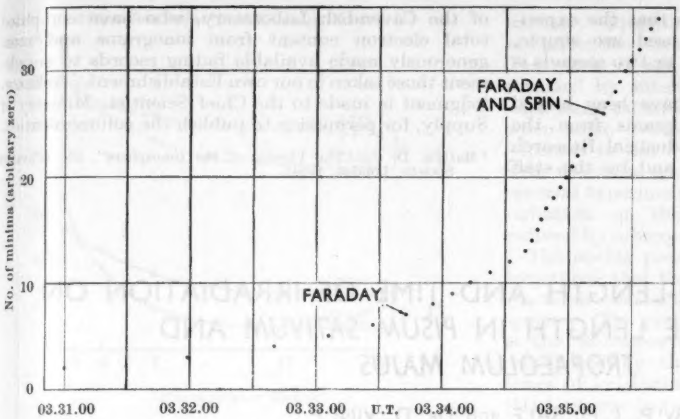
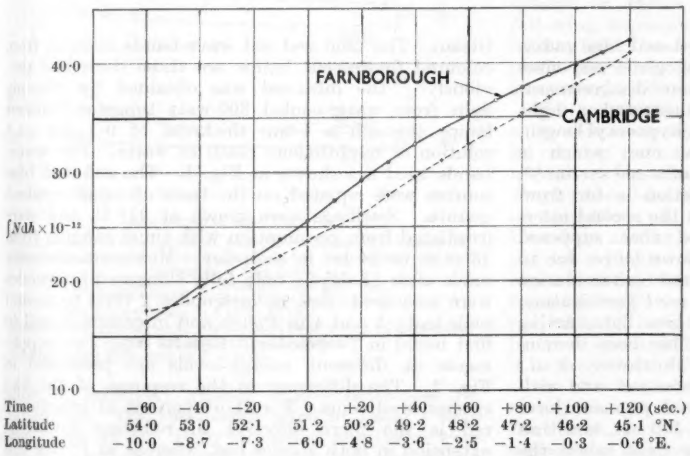


Fig. 1. No. of minima on the fading record taken on October 19, 1957. 40 Mc./s. signal



Time	−60	−40	−20	0	+20	+40	+60	+80	+100	+120 (sec.)
Latitude	54·0	53·0	52·1	51·2	50·2	49·2	48·2	47·2	46·2	45·1 °N.
Longitude	−10·0	−8·7	−7·3	−6·0	−4·8	−3·6	−2·5	−1·4	−0·3	−0·6 °E.

Fig. 2. $\int N dh$ plotted as a function of position for Farnborough and Cambridge observations

satellite moves through this point to any point on the trajectory. From this number of rotations the electron content of that part of the ionosphere between observer and satellite can then be calculated for various points along the trajectory, and hence the variation of the electron content along a slice of the ionosphere can be determined.

This method has been used to interpret fading records of *Sputniks* 1 and 2 taken at Farnborough and Cambridge when the satellites were nearly overhead and at heights above the F_2 maximum of the ionosphere. The resulting values of electron content could be checked against those deduced from ionograms taken at Slough and Inverness. A comparison of the results is shown in Table 1.

On any one transit the satellite did not pass over both Inverness and Slough. The vertical-incidence results were therefore compared with observations when the satellite was at the corresponding geomagnetic latitude. Geomagnetic co-ordinates were chosen because the data for the critical frequency of the F_2-region over the Earth's surface correlate well with them[1]. Table 1 shows this comparison where n_s, Farnborough and Cambridge, relate to satellite observations, and n_s, Slough and Inverness, to vertical-incidence soundings. At Slough and Inverness n_s was estimated assuming a block of ionization of density $N_m F_2$ extending from the height of the F_2 maximum ($h_m F_2$) up to the heights of the satellite, h_s.

For the 03.30 transits of *Sputnik* 1 on October 18 and 19, 1957, the height of the satellite was only some 50 km. above $h_m F_2$, and the estimated value of n_s (Slough and Inverness) should be only slightly larger than the values obtained from observations of the satellite. This conclusion is supported by the experimental results shown in Table 1. The slight discrepancies that arise can be attributed to errors in height of about 10 km. in either the satellite height or $h_m F_2$.

In the case of the 14.00 transit of *Sputnik* 2 on November 7, 1957, the height of the satellite was well above $h_m F_2$ (about 200 km.). The value of n_s obtained from the observations of the satellite would be expected to be markedly less than that deduced from the vertical-incidence soundings, assuming a block of ionization above $h_m F_2$. The value of n_s (Slough) compared with the satellite observations made at Farnborough and Cambridge is consistent with the above view. In the case of this high transit, the ionosphere explored when the satellite was at the geomagnetic latitude of Inverness was a region south of this latitude. It is shown in Fig. 2 that the electron content decreases towards the north, so that the proper value of n_s (Farnborough and Cambridge), to compare with n_s (Inverness), should be smaller than the value given in Table 1. The satellite observations made on November 7 are therefore consistent with the vertical-incidence soundings.

A comparison of the values of electron content deduced from simultaneous observations made at Farnborough and Cambridge is shown in Fig. 2. At the ends of the transit where the ray paths would pass through the same part of the ionosphere on the way to either base, the measured values are in good agreement. The difference in values in mid-transit probably results from the fact that the rays to Farnborough and Cambridge then follow different paths, with the Cambridge path north of the Farnborough path.

The results presented here show good agreement for observations at different stations, and also agreement with estimates made by an independent method. We conclude, therefore, that the method of analysis is reliable, and further observations along these lines are in progress. The method provides a way of determining horizontal variations in the ionosphere both above and below the maximum of

ionization, and has the advantages that the experimental technique and apparatus used are simple, while timing accuracy of about one or two seconds is sufficient.

In carrying out this work we have been helped greatly by the provision of ionograms from the Department of Scientific and Industrial Research stations at Slough and Inverness, and by the staff of the Cavendish Laboratory, who have computed total electron content from ionograms and most generously made available fading records to supplement those taken in our own Establishment. Acknowledgment is made to the Chief Scientist, Ministry of Supply, for permission to publish the communication.

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EFFECTS OF WAVE-LENGTH AND TIME OF IRRADIATION ON INTERNODE LENGTH IN *PISUM SATIVUM* AND *TROPAEOLUM MAJUS*

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THE relative effectiveness of red and blue radiation in controlling photomorphogenic responses of plants has been the subject of some disagreement. Workers at Beltsville have demonstrated a light reaction governing internode length, hypocotyl length, leaf expansion, seed germination, etc., which is mediated through a reversible red/infra-red system[1,2]. However, the effect of blue radiation is far from clear. Downs *et al.*[1], working with the second internode of *Phaseolus*, have claimed that supposed effects of blue radiation can be shown to be due to the relative levels of red and infra-red contamination in the source of blue light. For seed germination, however, an unequivocal effect of blue light, acting in the same direction as infra-red, has been demonstrated by Flint and McAlister[3]. Borthwick *et al.*[4] have suggested that both the infra-red and red-absorbing forms of the red/infra-red pigment have some absorption in the region 400–500 mμ, and that germination is promoted by blue light when the pigment is in the red-absorbing form and inhibited by blue light when the pigment is in the infra-red-absorbing form. The results of the Beltsville group have come mainly from short-term irradiation experiments with etiolated material of *Pisum*, *Phaseolus* and *Hordeum* grown in the dark. Workers in Europe have suggested that the relative effectiveness of blue and red radiation in determining final internode lengths varies according to the experimental technique, particularly the intensity of the light used and the duration of the illumination period[5]. Previous reports[6] from this laboratory have shown, however, that different results are obtainable when different species are given similar conditions of irradiation; shortest internodes occurred in red in *Pisum* and *Calendula*, and in blue in *Lycopersicon* and *Lactuca*. These relative differences in response held over a range of intensities, but very low intensities could not be used for the small-seeded 'blue-sensitive' species. However, the relatively large-seeded species *Tropaeolum majus* has been found to develop shorter internodes in blue than in red light and, accordingly, it has now been possible to measure the responses of the 'red sensitive' and 'blue sensitive' species over a much wider range of irradiances.

The plants used in the investigation were *Pisum sativum* Meteor and *Tropaeolum majus* Double Orange Gleam. The blue and red wave-bands isolated from coloured fluorescent lamps are those described previously[6]; the infra-red was obtained by filtering light from water-cooled 300-watt tungsten-filament lamps through a 1-cm. thickness of 0·1 per cent solution of naphthalene black in water. The wave-bands used are shown in Fig. 1. The red and blue sources were equated on the basis of equal incident quanta. Seedlings were grown at 21° C. and were irradiated from germination with times ranging from 15 min. to 24 hr. in each day. Measurements were made after 14 days; only fully elongated internodes were measured, that is, internodes 2 (first to second scale leaf), 3 and 4 in *Pisum* and internode 1 (soil to first node) in *Tropaeolum*. Results from two experiments at different energy-levels are presented in Fig. 2. The difference in the response of the two species is obvious. For short periods of irradiation red is the more effective in reducing internode extension in both species but, whereas in *Pisum* this effect is maintained for all times of irradiation, in *Tropaeolum* the relative effectiveness of red and blue radiation is reversed for times of irradiation of more than about 1½ hr.

In view of the red/infra-red system cited above, the effect of the near infra-red region on these responses was investigated. Times of irradiation of 4 hr. were chosen to bring the responses into the

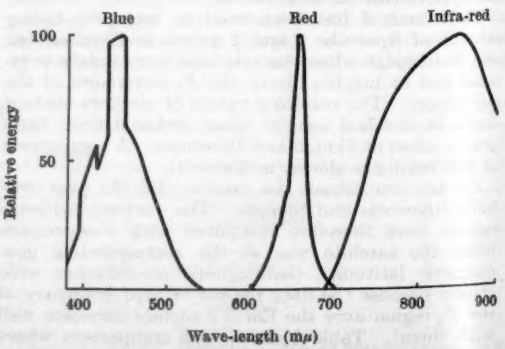

Fig. 1. Relative wave-length distribution of light sources

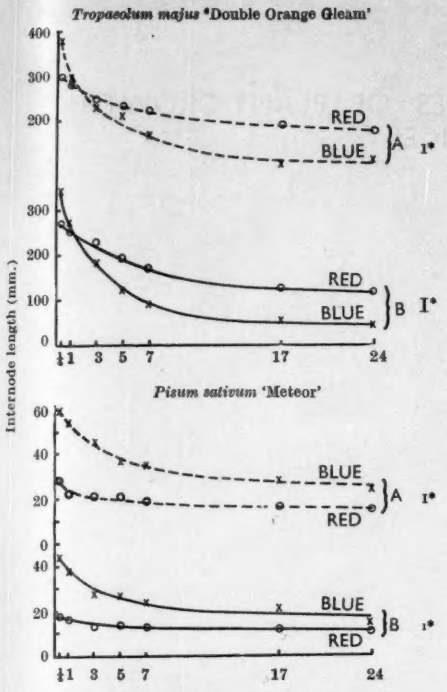

Fig. 2. Effect of irradiation on final internode length. Values for *Pisum* are means for internodes 2–4, for *Tropaeolum* are for internode 1 (see text)

(*A*) Energy-levels { Blue 900 ergs cm.$^{-2}$ sec.$^{-1}$ / Red 900 × 0·68 ergs cm.$^{-2}$ sec.$^{-1}$

(*B*) Energy-levels × 5 those in (*A*)

Dark values: *Pisum* (*A*) 81·2 mm., and (*B*) 66·0 mm.; *Tropaeolum* (*A*) 556 mm., and (*B*) 593 mm.

* Significant difference at $P = 0{\cdot}05$

region of relatively greater blue effectiveness for *Tropaeolum*. The treatments listed in Table 1 were given every 24 hr. Fully expanded internodes were measured as before after 12 days and the results (mean of two experiments) are given in Table 1. Although differing in their response to blue or red light at these times of irradiation, the two species behaved in essentially the same manner with regard to the effects of one wave-band of irradiation on another. Preliminary treatment with infra-red did not affect the response to either blue or red radiation. The effect of red radiation was almost completely reversed by subsequent exposure to infra-red even with these rather long preliminary exposures to red, when some irreversible growth responses might be expected to have occurred (namely, 4 hr. compared with the usual exposure times to red of 2–5 min. in reversal experiments of this kind). The effect of blue radiation, on the other hand, was only slightly reduced by subsequent irradiation in the near infra-red.

Table 1

Irradiation treatments			Length* of fully expanded internode (mm.)	
4 hr.	4 hr.	16 hr.	*Pisum*†	*Tropaeolum*‡
Infra-red	Dark	Dark	54·0	295·4
Blue	Dark	Dark	27·7	135·5
Infra-red	Blue	Dark	26·4	125·0
Blue	Infra-red	Dark	35·1	156·5
Red	Dark	Dark	13·6	188·0
Infra-red	Red	Dark	15·7	173·5
Red	Infra-red	Dark	42·5	250·2
Blue	Red	Dark	12·5	84·3
Red	Blue	Dark	19·2	123·0
Blue	Blue	Dark	23·8	96·8
Red	Red	Dark	11·9	162·0
Significant difference at $P = 0{\cdot}05$			5·9	20·6

* Means from two experiments.
† Mean of internodes 2–4.
‡ Internode 1.

Energy-levels { blue, 5,650 ergs cm.$^{-2}$ sec.$^{-1}$ / red, 5,650 × 0·68 ergs cm.$^{-2}$ sec.$^{-1}$ / infra-red, 4,160 ergs cm.$^{-2}$ sec.$^{-1}$

The results presented are in accordance with the hypothesis that there are at least two photochemical reactions controlling internode expansion in these species. The preliminary low-intensity reaction (I) is the well-known red/infra-red reversible reaction established by the Beltsville workers. At longer times of irradiation in *Tropaeolum* a second system (II) becomes more important; this latter system is more sensitive to blue radiation and is not reversible by infra-red. The effects of these two reactions appear to be additive. The presence of the latter system in *Pisum* is indicated by the fact that, following exposure to blue, infra-red has relatively little effect; however, at all the times of irradiation used, internode elongation was more effectively suppressed in *Pisum* by red than by blue light.

The treatment blue–red (Table 1) is of interest since this treatment is more effective than red–blue in causing reduction in internode length, although quantitatively the treatments are the same. In *Tropaeolum*, blue–red is more effective than red–red or blue–blue; in *Pisum*, it is more effective than blue–blue and as effective as red–red. These results can be explained if we assume that, in addition to any effect in 'reaction II', some blue light in the region 400–500 mμ is absorbed by both forms of the red/infra-red reversible pigment, and that its action in this system will depend on whether the pigment is in the red- or infra-red-absorbing form at the start of the blue irradiation (as suggested for the promotion and inhibition of seed germination in lettuce by Borthwick *et al.*[4]). At the end of the 16-hr. dark period most of the red/infra-red pigment will be in the red-absorbing form. Then, in the blue–red treatment, during the blue irradiation there will be a positive effect in 'reaction II' and some of the red/infra-red pigment will be converted to the infra-red absorbing (that is, active form); the subsequent exposure to red will increase this conversion. Conversely, in the red–blue treatment, most of the pigment will be in the infra-red-absorbing form at the end of the irradiation with red light and subsequent exposure to blue will tend to reverse the red effect; there will, however, be a positive effect of blue in 'reaction II'. There is no measurable infra-red contamination present in the source of blue light used in these experiments.

Further experiments are in progress on the time/intensity reciprocity in these responses.

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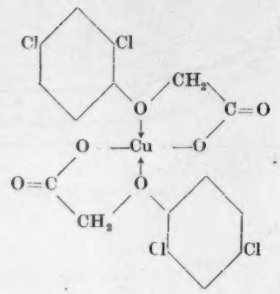
METAL-CHELATING PROPERTIES OF PLANT-GROWTH SUBSTANCES

THE suggestion that plant-growth substances act by virtue of their ability to chelate metal ions has received some support from work reported recently by Cohen *et al.*[1] on the formation of copper complexes by indoleacetic acid and naphthalene-α-acetic acid. We wish to report experiments, prompted by their communication, which can be taken to contradict this general hypothesis and disagree in detail with some of the results quoted. We have formed and measured the stability constants of copper complexes of phenoxyacetic acids which have a wide range of growth activity. On the suggested hypothesis the copper complexes should show a similar wide range of stability constants, but we have found them to be indistinguishable.

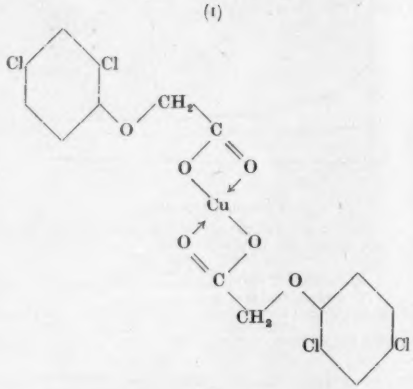
The copper complexes of the following acids have been studied: 2 : 4-dimethylphenoxyacetic acid, 4-chloro-2-methyl phenoxyacetic acid and 2 : 4-dichlorophenoxyacetic acid, and the analyses of the complexes are shown in Table 1. We have also made measurements of the copper complex of naphthalene-α-acetic acid for comparison with earlier work. Attempts to obtain evidence of the formation of copper complexes in this series with an acid/copper ratio of 1 : 1 failed. The complexes isolated under various conditions were shown to be hydrates of the (2 : 1 : acid : copper) compounds. After dehydration at 140° C. and 0·1 mm., the anhydrous salts were quite stable and did not absorb moisture from the air even after standing for a week. The optical densities of 50 per cent ethanolic solutions containing increasing ratios of acid/copper were determined at wave-lengths where the copper complex absorbs relatively more than the free acids. Plots of optical density against the ratio of acid/copper showed only one break in the curve, at an acid/copper ratio of approximately 2.

Whether or not the materials described above are true complexes in which the organic component is doubly bound to the metal may be questioned. The alternative would be to regard them as simple copper salts. In view of the stability of the anhydrous materials, the formulation as salts would only be valid if the copper had a co-ordination number of 2. The usual co-ordination number of 4 would be consistent with complex formation, involving either one acid and one other oxygen atom as in I or the two acid oxygen atoms as in II. The latter structure has been established for the copper derivatives of fatty acids[2], and we regard it as likely also to be correct in the phenoxyacetic acid series.

The stability constants of the copper complexes were determined by *p*H titration measurements[3] in



(I)



(II)

50 per cent ethanol. The values in Table 2 were calculated from measurements made in the absence of a strong acid, following what was apparently done by Cohen *et al.*[1]. The *pKc* values differ very little and the copper complex of the acid with the least growth activity is the most stable. Our value for the *pKa* of naphthalene-α-acetic acid (5·76) differs from theirs (4·6), so that the calculated *pKc* which critically depends on the determined *pKa* is also different from their value of 5·4. In so far as our value is higher, it indicates a slightly greater stability for the complex. The presence of a strong acid, however, is theoretically important to the method of *p*H titration[4] and Table 3 lists the constants determined in 50 per cent ethanol in the presence of a strong acid (10^{-5} *M* nitric acid). It will be seen that the stability constants of the five copper complexes differ by insignificant amounts; indeed, the second figure is scarcely reliable, considering the assumptions made

Table 1

Copper complex	Found C	Found H	Formula for dehydrated complex	Calculated C	Calculated H	Hydrate	M.p. (°C.)
2 : 4-Dimethylphenoxyacetic acid	56·93	5·14	$C_{20}H_{22}O_6Cu$	56·94	5·26	$2H_2O$	235–6 (decomp.)
2-Methyl-4-chlorophenoxyacetic acid	46·51	3·53	$C_{18}H_{16}O_6Cl_4Cu$	46·73	3·49	$2H_2O$	247–9 (decomp.)
2 : 4-Dichlorophenoxyacetic acid	38·14	2·25	$C_{16}H_{10}O_6Cl_4Cu$	38·18	2·00	$5H_2O$	254–6 (decomp.)

Table 2

Copper complex	Stability constants of copper complexes based on measurements in the absence of a strong acid			
	pKa	pK_1	pK_2	pKc
Naphthalene acetic acid	5·76	5·1	4·5	9·6
2 : 4-Dimethylphenoxyacetic acid	4·54	4·6	4·2	8·8
2-Methyl-4-chlorophenoxyacetic acid	4·36	4·3	4·0	8·3
2 : 4-Dichlorophenoxyacetic acid	4·20	4·3	3·8	8·1

pKa is the equilibrium constant of the acid; temperature, 21° C. (pK_1 and pK_2) = pKc, the chelate stability constant of the reaction: $2A^- + Cu^{++} \rightleftharpoons (A)_2Cu$; all determinations made in 50 per cent ethanol.

Table 3

Copper complex	Stability constants of copper complexes based on measurements made in the presence of 10^{-2} M nitric acid				Growth activity of acid (dose in μgm. needed to give 50 per cent leaf repression)
	pKa	pK_1	pK_2	pKc	
Naphthalene-α-acetic acid	5·75*	5·2*	4·5*	9·7*	0·2 ± 0·1
2 : 4-Dimethylphenoxy-acetic acid	4·53	4·6	3·9	8·5	28 ± 2
2-Methyl-4-chlorophen-oxyacetic acid	4·39	4·4	3·8	8·2	0·15 ± 0·02
2 : 4-Dichlorophenoxy-acetic acid	4·19	4·3	4·2	8·5	0·04 ± 0·005

* Identical values obtained in independent experiments; temperature, 25° C.; all measurements in 50 per cent ethanol.

in calculating the pKc. Similar disagreement with the recorded pKc for indoleacetic acid – copper complex has been reported recently by Recaldin and Heath[5], although it is not clear what conditions were used for their measurement.

Parallel with the measurements of the stability of the copper complexes, we have made quantitative estimates of the growth-regulating activity of each of these growth substances by the method of Brown and Weintraub[6]. In the last column of Table 3 are listed the quantities of each acid required to depress the growth of the first trifoliate leaf of a *Phaseolus* seedling by 50 per cent. The percentage repression in this test is linearly related to the logarithm of the dose, and the figures for 50 per cent repression are inversely related to the growth activity. It will be seen that 2 : 4-chlorophenoxyacetic acid is almost a thousand-fold more active than dimethylphenoxy-acetic acid, though its ability to bind copper is not measurably different.

We therefore suggest that in spite of some evidence in the literature for a relation between metal-complexing capacity and plant-growth activity, the properties by which certain molecules are specifically able to act as growth regulators still remain a mystery.

Our thanks are due to the directors of Imperial Chemical Industries of Australia and New Zealand for permission to publish this communication, and to Mr. D. Fieldhouse, Mrs. M. Markus and Mrs. V. Zangari for technical assistance.

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In putting forward the hypothesis that plant-growth substances might act as such through their metal complex-forming activities[1,2] we did not suggest that no other properties of the molecules concerned could modify their effects upon growth. We specifically raised the question of rate of penetration, and in view of the very various growth-promoting activities of the different agents tried (a variation by no means parallel with their metal-complexing capacities) we had in mind the possibilities that steric hindrance or toxicity might also be modifying the amount of growth promotion obtained.

One might well hesitate to accept that the 'growth-regulating activity' of 2 : 4-dichlorophenoxyacetic acid is almost a thousand times as great as that of dimethylphenoxyacetic acid on the basis of an assay test depending on the inhibition of growth, such as that used by Armarego *et al.* (preceding communication), but other work[3] suggests that their growth-promoting activities in the *Avena* cylinder test are in a similar ratio. Even if this is not, in fact, due to a difference in penetration, the binding of copper or some other metal may yet be concerned with the spatial configuration of the sites favouring one acid rather than the other.

The growth-promoting activity of ethylenediamine tetraacetate has been variously reported as similar to that of indoleacetic acid[1,4], or as much less[5,6], according to the techniques and plant material used; with the strain of wheat used by Fawcett *et al.*[6], and using their technique, we have been able to reproduce their results. However, even if the growth-promoting activity of these two agents be accepted as of the same order of magnitude, the fact remains that their *in vitro* complexing activity differs vastly. It is, in fact, surprising that so powerful a chelating agent as ethylenediamine tetraacetic acid does not disrupt the chemical organization of the cell at many sites; the growth-promotion obtained may be the net result of stimulatory and inhibitory processes due to competition with various chelating agents in some such way as that suggested by Burström and Tullin[7].

Evidence seems to be accumulating that known natural and synthetic growth substances can form complexes *in vitro*[8,9], but more important than this is the question whether they do so in controlling growth in the living plant. Some of the evidence for this was very briefly reported in the communications cited[1,2] and the results of these and other experiments will be published in detail as soon as possible.

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LETTERS TO THE EDITORS

PHYSICAL SCIENCES

A New Ionospheric Phenomenon

In recent years, there have been reports[1–4] of sporadic radio-frequency radiation associated usually with auroral activity. However, it has always been difficult to establish an unambiguous identification because similar effects could be caused by man-made noise or interference. The special programme initiated at Jodrell Bank in October 1958 to study these unusual radiations provided a high degree of flexibility by using five separate total-power receivers, all on slightly different frequencies near 80 Mc./s., to monitor continuously various sectors of the sky. At present, two of the aerials are directed at 30° elevation above the northern horizon, one is directed at the zenith, one at 30° above the southern horizon and the other is rotated continuously so that Cassiopeia 23*N*5*A* is always in the beam. Three of the equipments are at Jodrell Bank and the others are on individual sites about 1 km. away. This experimental arrangement allows easy discrimination against localized interference at any one site, and against distant narrow-band radio signals.

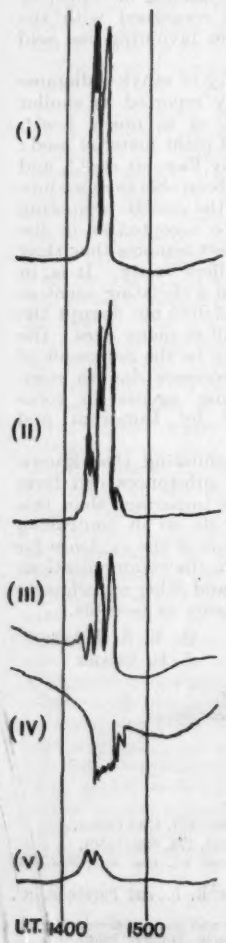

Fig. 1. Records from (I) northerly, (II) northerly (low sensitivity), (III) continuously following Cassiopeia, (IV) zenithal and (V) southerly aerials

Normal records from the equipments show the expected diurnal variation due to the galactic background and, in addition, the southern aerial records radio bursts and noise storms of solar origin. However, during the period January 3–10, 1959, there occurred about ten instances of isolated increases in the noise-level recorded by some of the instruments, together with *simultaneous decreases* in the others[5]. A further very striking isolated event occurred on March 25, 1959, at about 1400 U.T. The records are reproduced in Fig. 1. It can be seen that the northerly channels (i) and (ii) showed very strong enhancements in the signal level. The amplitude of (i) was greater than 150 per cent of the diurnal change in galactic level, and the increase in (ii) was 400 per cent of the diurnal galactic change. The former instrument was driven off-scale while the latter was operating at reduced sensitivity. The apparatus continuously following Cassiopeia (iii) with its aerial directed at this time toward the north-west showed a strong enhancement of approximately 50 per cent of the diurnal galactic range. The zenith instrument (iv) recorded a marked decrease of at least 50 per cent of the diurnal range of galactic intensity, while the south (v) indicated a weak increase of about 25 per cent. Since the back-to-front rejection ratio of the south-looking Yagi aerial is about 1 : 10, it is probable that the apparent southerly increase is spurious in view of the very strong northerly signal. At the time of this event the Sun was actually in the beam of the south aerial, hence one may conclude that direct solar radio-frequency radiation was negligible.

The Jodrell Bank magnetometer revealed a change in the horizontal component of the Earth's field coinciding with these observations. The change of about 50 gammas is the most significant fluctuation in an interval of 4–5 hr. However, the significance, if any, of the magnetic crochet is masked by the presence of other fluctuations of comparable size both earlier and later in the day.

This event, with its simultaneous radio-frequency emission and absorption in different sectors, is more intense than any observed in the January 1959 series. However, it has the same essential characteristics. The suggestion has been made[5] that this phenomenon is caused by passage through the ionosphere of streams of charged particles of very high velocity presumably of solar origin. In the upper ionosphere such particles stimulate the generation of radio-frequency energy while at lower levels the result is a net absorption of the background signal. The absorption mechanism is rather well established, especially in polar regions, and may be attributed to the production of abnormally dense ionization in the lower ionosphere. However, very little is known of processes which can generate radio-frequency noise in our atmosphere. It is believed that the impact of charged particles, both of high velocity and high density, is consistent with the environment for production of Čerenkov-type radiation. Other processes are also being considered.

Events of the type described seem to be rare. It is significant that both the first event of the group in January already reported, and the isolated event of March 25, seemed to be the precursors of a period of intense solar activity with associated terrestrial events such as magnetic storms and auroræ. A preliminary investigation has failed to reveal any unusual solar or other terrestrial effects which may be associated with this phenomenon.

We wish to acknowledge the continued interest and enthusiastic support of Prof. A. C. B. Lovell in this research.

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Observations of Intense Ionization of Long Duration below 50 km. Altitude after some Strong Solar Flares

THE existence of ionized layers (the *C*-layers) in the lower and middle atmosphere (between 5 km. and 60 km. altitude) was suggested more than twenty years ago by several authors and such short-range echoes (20–50 km.) have also been reported in recent years. It seems doubtful, however, whether they were produced by ionization[1]. Landmark[2] has recently obtained a few echoes from a height of 65 km., which seem to have been of ionospheric origin. They appeared during periods of strong solar disturbances. Intense ionization at still lower heights was observed four times during the summer of 1958 at Kiruna Geophysical Observatory, Sweden.

The total non-deviative ionospheric absorption of 27·6 Mc./s. radio waves is being measured at the Observatory on a routine basis by means of a so-called 'riometer' (relative ionospheric opacity meter) designed by C. G. Little[3] at the Geophysical Institute, College, Alaska. The riometer is a self-balanced radio noise telescope using a vertical three-element Yagi antenna and having a so-called minimum detector, which makes measurements possible of the cosmic radio-noise level even in the presence of strong interference. The recorded cosmic-noise flux gives a relative measure of the overall absorption in the ionosphere.

On four occasions during the summer of 1958 very strong absorption was observed to start a few hours after the outbreak of solar flares of importance 3+. In three of these cases the maximum day-time absorption[4] exceeded 15 db. At the time of the first observation, July 7, 1958, it was midnight sun in Kiruna, but during the other three periods of observations, starting on August 16, 22 and 26 respectively, the shadow of the solid Earth reached from 62 km. on August 16 to 125 km. on August 26 above the Earth's surface at midnight.

The ratio of negative ion density to free electron number density is a function of the hour angle, as one of the processes determining the ratio is photo-detachment of electrons from negative oxygen ions, and thus the absorption is smaller during the night than during the day. The electron affinity of the oxygen molecule is approximately 1 eV., which means that the photons of almost the whole solar emission spectrum have sufficient energy for the photo-detachment reaction.

The riometer records for the nights of August 16–17, 22–23, 23–24 and 26–27 were all curves of the type shown in Fig. 1. During the evenings the absorption decreased to a minimal, and almost constant, night-time value, although the shadow of the solid Earth reached levels varying between 44 and 53 km. only (average of four values, 48·3 km.) above the surface of the Earth. Similarly, absorption started to increase again in the mornings when the shadow of the Earth had reached heights between 39 and 55 km. (average of three values, 48·3 km.). During the morning of August 24, a geomagnetic sudden commencement started before sunrise, and therefore no observations could be made.

In two of the seven cases the record is concave upwards (cf. Fig. 1) during the whole transition period between day-time and night-time conditions. In those cases the time of end and beginning respectively of the transition is estimated to be in error by only some ± 3 min. This corresponds to an uncertainty of height of less than ± 2 km. In the remaining five cases the uncertainty is about 5 km.

An explanation of the early arrival of the absorbing layer into the dark region may be that the effective shadow is not that of the solid Earth but of some layer in the atmosphere, for example, the ozonosphere. That explanation can, however, be ruled out by the following facts. It has been found at Stanford, California[5], that the intensity of reflexions on 325 kc./s. tends to increase when the solar rays tangential to the Earth strike the lower *E*-region, and the height of the lowest observed *E*-region reflexion on 100 kc./s. was found to drop appreciably at about the time when the region left the shadow of the solid Earth. These effects are attributed to electron photo-detachment, as in the cases under discussion here. Furthermore, the investigations of the distribution of ozone with height, using the eclipsed Moon as reflecting surface[6], are based on the fact that visible radiation, even in the Chappuis diffuse absorption bands of ozone, penetrates the Earth's atmosphere at the ozone-levels, reaches the eclipsed Moon and is reflected back to the spectrometer on the Earth's night-side. As all radiation of wave-lengths less than approximately 12,000 A. can produce free electrons by photo-detachment from O_2^- ions, the only remaining explanation of the fact that the illumination of the layers above the heights mentioned earlier does not result in any measurable radio-wave absorption in addition to that of the lower layers, seems to be that all but a negligible fraction of the ionization is situated below the heights in question. At such great depths in the atmosphere the absorption coefficient is in fact not increasing with depth as it is in higher levels, due to the fact that for very high values of the electron collision frequency the absorption decreases again. The transition-level is situated between 50 and 60 km.

If one takes into account the possible complete opacity of the lowest few kilometres of the atmosphere, the upper limits of the absorbing layers, given above, are not raised by more than the same small amount (a few kilometres).

The observations reported here support the hypothesis that the ionizing agent on the occasions under discussion was protons, emitted by the Sun during the solar flares (see ref. 4, Hultqvist, B.). The very low recombination coefficients found for this kind of ionization[7] may perhaps also be associated with the

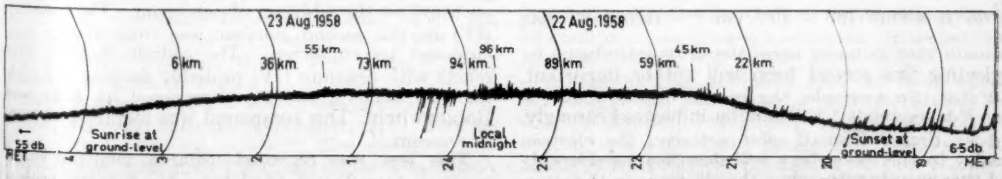

Fig. 1

fact that most of the ionization was situated within the ozonosphere, as is certainly the observed absence of magnetic effects of the ionization.

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Radio Reflexions from the Moon and Solar Corona

The ionospheric Faraday effect has recently been used[1,2] to determine the total electron content of the ionosphere. The rotation of the plane of polarization is assumed to be given by :

$$\varphi = 4{\cdot}72 \times 10^4 (f^{-2} B \cos\theta \sec\delta \int_{H_1}^{H_2} N \, dh) \qquad (1)$$

where the limits of integration correspond to the ionospheric region in which the electron density is large enough to make an appreciable contribution to the rotation (for notation see ref. 2).

On the other hand, the theory of 'whistlers', investigations on the solar corona[3] and also the researches on the first Russian satellite[4] have led to the assumption that there are some hundred electrons per c.c. in the space around the Earth. This necessitates modification of formula (1). If we assume a constant electron density N_0 along the path from the outer ionosphere ($r = r_E + H_2$) to the Moon ($r = r_M$) we have :

$$\varphi = 4{\cdot}72 \times 10^4 f^{-2} \left\{ B \cos\theta \sec\delta \int_{H_1}^{H_2} N \, dh + N_0 \int_{r_E + H_2}^{r_M} B \cos\theta \, dr \right\} \qquad (2)$$

or, to a good approximation :

$$\varphi = 4{\cdot}72 \times 10^4 f^{-2} B \cos\theta \left\{ \sec\delta \int_{H_1}^{H_2} N \, dh + \tfrac{1}{2} N_0 (r_E + H_2) \right\} \qquad (3)$$

With $(r_E + H_2) = 7{\cdot}4 \times 10^8$ cm. and $N_0 = 10^2$ cm.$^{-3}$, we get $3{\cdot}7 \times 10^{11}$ cm.$^{-2}$ for the second term, while $\int_{H_1}^{H_2} N \, dh$ is about 100×10^{11} cm.$^{-2}$. Hence we can estimate that in many cases the error introduced by neglecting this second term will not be important, and that, for example, the results obtained on the new F-layer model[5] will not be influenced strongly. But at times of small solar activity, the electron density in the ionosphere will decrease considerably and then we cannot neglect the electrons in the space around the Earth. In these and some other cases (for example, in the polar regions) we must take the revised formula (2) into consideration.

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Nature of Fluorescence of Uranium in Fused Sodium Fluoride

Although the property of fluorescence of uranium in sodium fluoride melts has been used to determine uranium quantitatively for the past 25 years, it is not known in what form the uranium exists in these melts. It has been the belief that the fluorescence in fused salts is due to a uranyl compound, but the evidence is not conclusive[1,2]. The intensity of fluorescence of the melts is so sensitive to slight variations in the fusion process that it is necessary to standardize all manipulations to obtain reproducibility. The cause of this sensitivity has not been explained. In an attempt to explain it several different uranium compounds were fused in different atmospheres. The intensities of fluorescence of the melts as well as fluorescence and absorption spectra were measured.

All the uranium compounds used for the experiments were made in this Laboratory. A sample of uranium tetrafluoride was also obtained from Harwell. A very high standard of purity is required as many impurities quench the uranium fluorescence. The sodium fluoride used was 'Baker analysed' and was specially prepared for use on the fluorimeter. This brand of flux was found to be entirely free of sodium carbonate, which is useful at times but not essential, as sodium fluoride–sodium carbonate flux mixtures are often used. Sodium fluoride decomposes during fusion to form small quantities of sodium carbonate when moisture and carbon dioxide are present in the atmosphere[3].

UO_3, U_3O_8, UO_2, UF_4 and $UO_2(NO_3)_2$ were fused with sodium fluoride in air at 1,160° C. and all melts fluoresced strongly. The high fluorescence of UF_4 was unexpected since uranium (IV) compounds have not been known to fluoresce. A test was devised to determine the valency of the uranium in the melt, using the property that cupferron precipitates uranium (IV) but not uranium (VI) in 10 per cent sulphuric acid solution.

Two separate portions of a solution containing uranium (IV) in 10 per cent sulphuric acid were treated with cupferron. Sodium fluoride had first been added to one portion, when a powdery green precipitate had formed. No further precipitation occurred on the addition of cupferron. The uranium (IV) in the second portion was immediately precipitated by cupferron. The sodium fluoride thus reacts with uranium (IV) probably forming a double fluoride, namely, $NaUF_5$ as reported by Katz and Rabinowitch[3]. This compound was found to be non-fluorescent.

This test was repeated using a melt in which uranium tetrafluoride had been fused in air with a low-melting mixture of sodium fluoride and sodium

carbonate. When it was dissolved in 10 per cent sulphuric acid no green powdery precipitate settled out and the uranium was also not precipitated by cupferron. From this it was concluded that the uranium is oxidized to the hexavalent state during the fusion process.

The fluorescent species resulting from fused uranium tetrafluoride is therefore not tetravalent as the compounds examined by Zachariasen[2] appear to be.

The fusion of all the uranium compounds was repeated using an argon atmosphere in a tube furnace. All melts were fluorescent but intensities were much reduced. When oxygen was added to the atmosphere the intensities of the argon-fused melts increased in most cases. This seems to indicate that oxygen is required for high fluorescence. The initial fluorescence in argon, particularly of uranium tetrafluoride, appears to be due to the presence of minute quantities of oxygen which diffuse through the walls of the rubber tubing. This is very difficult to eliminate.

When sodium hydrogen fluoride is used as a flux in air with U_3O_8, UF_4 and $UO_2(NO_3)_2$, the usual high fluorescence is obtained, but when fusions are made in argon virtually no fluorescence occurs. This is ascribed to the action of hydrogen fluoride that is evolved from the flux on heating. Katz and Rabinowitch report that very small quantities of hydrogen fluoride are sufficient to prevent the oxidation of $UF_4.H_2O$ during dehydration at 400° C. Hydrogen fluoride also prevents the formation of oxy compounds[2].

The fluorescence spectra of the melts were examined and several variations were measured. The spectrum extended from approximately 5100 to 6500 A. Under certain conditions the lowest band limit ended at 5350 A. The main variations occurred in the violet region where bands appeared and disappeared according to the atmosphere, concentration, time of fusion and rate of cooling.

The spectra of uranyl salts do not vary in this manner. They consist of 6 or 7 bands extending from approximately 4600 to 6400 A. which shift nearer to or farther from the red region, depending on the composition of the salt.

The variation in the spectrum of the sodium fluoride – uranium melts appears to be the cause of the inconsistent intensities. It is not known what this variation is due to but it is suspected that an oxygenated uranium compound, with a variable constitution, is responsible.

The absorption spectra of argon-fused $UO_2(NO_3)_2$, UF_4 and U_3O_8 were measured on the Beckman *DU* spectrophotometer. Clear disks were obtained only when carbonate-free sodium fluoride was used. Melts fused in air became opaque due to the formation of sodium carbonate during fusion. These could not be measured on the spectrophotometer. The spectra of the pale yellow disks were measured against a blank of clear, colourless fused sodium fluoride.

In each spectrum maximum absorption occurred at 290 mμ. Absorption decreased steadily to 410 mμ and levelled off. No further peaks occurred, but each has a plateau from 350 to 360 mμ. Two of these curves are shown in Fig. 1.

Uranyl salts all have absorption peaks[4] in the region 400–500 mμ, while uranium (IV) absorbs in an entirely different manner[5]. The spectra of the fused melts resemble those of diuranates, which absorb strongly at 320 mμ with no further peaks occurring at higher wave-lengths[6].

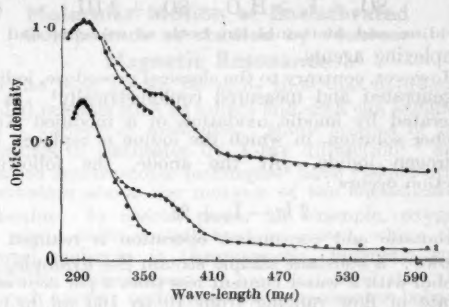

Fig. 1. Top, absorption spectrum of argon-fused uranyl nitrate using hydrogen and tungsten lamp sources; bottom, absorption spectrum of argon-fused uranium oxide (U_3O_8) using the same light sources

Uranyl salts are not stable at high temperatures. When ignited above 350° C. they decompose to form oxides. In the presence of a flux such as sodium fluoride which melts at 997° C. this decomposition must also occur, so that by the time the flux is molten the uranium is probably all present as U_3O_8. This then reacts with or dissolves in the molten sodium fluoride.

The fluorescent species formed does not resemble any of the parent compounds. It appears that any uranium compound can be fused with sodium fluoride at 1,200° C. to form the same or similar species provided that oxygen, moisture and several other constituents are present in the atmosphere. The best atmosphere for this is air.

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CHEMISTRY

An Automatically Operating, Coulometric Titration Method for continually measuring Small Amounts of Water in Fluids

Several batch-wise methods for the determination of small amounts of water are known. However, most of these methods lack general applicability.

In the method described here we have applied the well-known and versatile principle of the Karl Fischer titration. According to this titration procedure, the water to be determined reacts with the so-called Karl Fischer solution, a mixture of sulphur dioxide, iodine, pyridine and methanol. The simplified net reaction can be represented as :

$$SO_2 + I_2 + H_2O \rightarrow SO_3 + 2\,HI \qquad (1)$$

Pyridine and methanol act both as solvents and as complexing agents.

However, contrary to the classical procedure, iodine is generated and measured coulometrically[1]. It is generated by anodic oxidation of a modified Karl Fischer solution, in which the iodine is replaced by hydrogen iodide. At the anode, the following reaction occurs :

$$2\,I^- \rightarrow I_2 + 2e \qquad (2)$$

Automatic and continuous operation is realized as follows : a constant sample stream (for example, an alcohol with a water content less than 2 per cent and a rate of flow ranging from 10 to 100 ml./hr.) is supplied to the coulometric cell (see Fig. 1). In this cell, iodine is generated at the platinum wire electrode, *w.e.* The anode compartment is separated from the cathode compartment by a sloping, sintered glass disk to avoid redox cycles. The magnitude of the generating current i_g is controlled by a combination of an amperometric system and an electronic control system, so that sufficient and no excess iodine is generated for reacting with all of the water entering the cell. In this case the generating current —which can be recorded in simple manner—is a linear function of the water content of the sample. From Faraday's law, 1 μgm./sec. of water supplied to the cell needs a generating current of 10·72 m.amp., provided the current—as well as the titration—efficiency of the electrochemical reaction is 100 per cent. It appears that 100 per cent current efficiency is obtained when the current density does not exceed a critical value, dependent on the conditions of flow. The titration efficiency is—as in the classical titration —not 100 per cent ; it is, however, constant, the actual value being dependent upon the composition of the electrolyte.

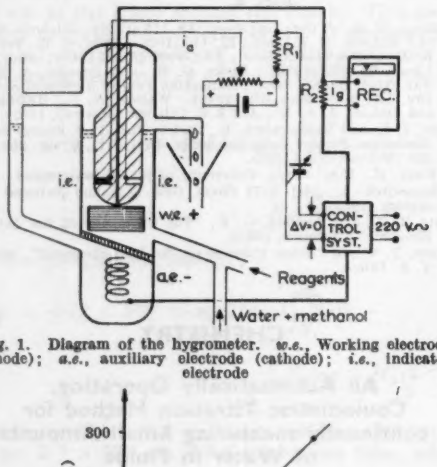

Fig. 1. Diagram of the hygrometer. *w.e.*, Working electrode (anode); *a.e.*, auxiliary electrode (cathode); *i.e.*, indicator electrode

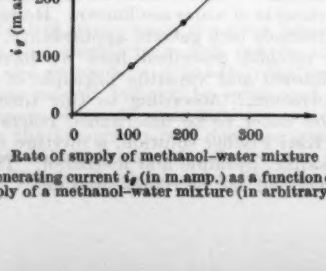

Fig. 2. Generating current i_g (in m.amp.) as a function of the rate of supply of a methanol–water mixture (in arbitrary units)

The amperometric system consists of two platinum electrodes, placed in an equipotential surface and provided with a potential difference of 25 mV. The generating current is so controlled that when the amperometric current i_a exceeds a fixed value of some microamperes (for example, when the water content of the sample decreases), the generating current decreases, etc. Environmental moisture must be excluded rigidly.

A laboratory apparatus built according to the above principles has worked satisfactorily. A typical graph is reproduced (Fig. 2) showing the generating current as a function of the rate of supply of a methanol–water mixture of a definite composition, the speed being expressed in an arbitrary measure.

While preparing this communication, our attention was directed to a recent paper by Meyer and Boyd[1], who have applied the same principle to the batch-wise titration of water.

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Molecular Symmetry in *Cyclo*pentadienyl Thallium and Some Related Substances from their Microwave Spectra

It was recently found in this laboratory[1] that the symmetry of *cyclo*pentadienyl nickel nitrosyl is simply and unequivocally shown by its microwave spectrum. We have now applied the same method to demonstrate the symmetries of *cyclo*pentadienyl thallium, *cyclo*pentadienyl manganese tricarbonyl and benzene chromium tricarbonyl. The manganese compound had sufficient vapour pressure at room temperature, but the other materials were studied in a Stark modulation spectrometer operated at about 100° C.

Several transitions of each substance were measured between 20 and 40 Gc./s. The spectra are those of strictly symmetrical-top molecules, with values of B, in Mc./s., as follows : *cyclo*pentadienyl thallium-205, 1465·10 ± 0·05 ; *cyclo*pentadienyl thallium-203, 1467·98 ± 0·05 ; *cyclo*pentadienyl manganese tricarbonyl, 826·5 ± 0·5 ; benzene chromium-52 tricarbonyl, 729·8 ± 0·5. Fine structures were not resolved, but spectra of molecules in excited vibrational states occurred, especially for the carbonyls. For these, B refers to the state showing the strongest absorption, but it is not certain that this is the ground-state. For *cyclo*pentadienyl thallium the lines are sharper, and those due to vibrationally excited molecules occur at lower frequencies than the ground-state absorptions. Separate series of lines due to *cyclo*pentadienyl thallium-205 and thallium-203 are recognized from their pattern and intensities.

The spectra prove that the *cyclo*pentadienyl compounds have axes of C_{5v} symmetry perpendicular to the C_5- and H_5-rings, and passing through the metal atoms. In benzene chromium tricarbonyl the corresponding axis is doubtless one of C_{6v} symmetry but, so far as microwave spectra can show, the symmetry might be merely C_{3v}. The tricarbonyl groups $(CO)_3$, when present, have C_{3v} symmetry about these same axes. The structure of *cyclo*pentadienyl thallium is thus the 'half-sandwich' type favoured by Cotton and Reynolds[2] on the basis of infra-red spectra. The tricarbonyls each have eight structure parameters, and detailed discussion is not profitable ; but the values of B are consistent with dimensions expected

from the study of related molecules. *Cyclo*pentadienyl thallium has only four structure parameters and, while a complete determination is not yet possible, it is profitable to examine carbon–carbon and carbon–thallium distances which are permitted for all reasonably likely values of the parameters affecting the hydrogen atoms. The simplest preliminary assumption is that the C_5H_5 system lies in one plane. With this assumption only, the distance from the thallium to the centre of the ring is found as 2·41 ± 0·01 A. If, in addition, we take d_{CH} as 1·08 A., we obtain $d_{CC} = 1{\cdot}43 \mp 0{\cdot}02$ A. and $d_{CTl} = 2{\cdot}705 \mp 0{\cdot}005$ A. The errors stated are those which could arise from possible inaccuracy in measurement of the small thallium isotopic shift; effects of changes in zero-point energy have been neglected. A variation in the value assumed for d_{CH} of ± 0·02 A. leads to additional changes of only ∓ 0·004 A. in d_{CC} and ∓ 0·002 A. in d_{CTl}. We have also investigated the effects of allowing the CH bonds to be bent out of the C_5-plane. An angular displacement of 5 deg. away from the thallium atom in these bonds leads to an increase of 0·025 A. in d_{CC}, and a decrease of 0·007 A. in the distance from the thallium to the centre of the ring. The distance d_{CTl} is increased by only 0·003 A. Its effect on d_{CC}, and general considerations, suggest that such bending is of small magnitude.

On such a basis, the C_5-ring appears to be similar in size to that found in ferrocene[2], with the possibility of being somewhat larger. The carbon–thallium distance is certainly longer than anticipated by Cotton and Reynolds[2], but there is very little evidence on which to base a prediction. From available data[4], a length of 2·4 A. would not seem impossible for a simple Tl–C linkage, and a greater length is naturally expected in a complex of this type. The distance found, however, coupled with the estimates of overlap integrals by Cotton and Reynolds[2], strengthens the evidence that ionic binding is important in this linkage. From microwave data for metallic halides as ion-pairs, Honig, Mandel, Stitch and Townes[5] tabulate ionic radii for such systems. From these and similar results for the largely ionic gaseous thallous halides[6], the corresponding radius for Tl^+ must be close to 1·0 A. The structure $C_5H_5^-$ Tl^+ would thus require the $C_5H_5^-$ ion to have an axial radius of about 1·4 A., which does not seem unreasonable. While the structure can scarcely be entirely ionic, a considerable ionic character would not be out of keeping with the findings for the thallous halides.

A detailed report, with fuller discussion, will appear elsewhere. We thank Prof. G. Wilkinson and Dr. M. C. Whiting for gifts of materials, the Royal Society for the loan of equipment and Prof. J. C. Robb for his interest in this project. Two of us (J. K. T. and A. P. C.) acknowledge maintenance grants from the Department of Scientific and Industrial Research.

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Molecular Motion of Enclathrated Compounds as studied by Nuclear Magnetic Resonance

It has been known for some time that hydrogen-bonded crystals such as quinol and urea can enclathrate a variety of molecules. Many studies of the structures of clathrates by X-ray diffraction[1–4] and infra-red spectroscopic techniques[5] have yielded little information about the motions of the enclathrated molecules. In special cases, for example, oxygen and nitric oxide enclathrated in quinol, where the trapped molecule is paramagnetic, magnetic susceptibility measurements[6–8] have indicated that the trapped oxygen and nitric oxide molecules behave rather like free gas molecules.

For enclathrated molecules which are not paramagnetic it seemed that nuclear magnetic resonance spectroscopy would provide information about the motion of the trapped compound. We wish to report some preliminary results which we have obtained by studying the proton magnetic resonance signals from certain enclathrated hydrocarbons.

Though a wide range of clathrates are currently under investigation by a variety of methods, we shall give here only some results for magnetic resonance studies on urea hydrocarbon adducts, the hydrocarbon ranging from C_{7-13}, C_{16} and C_{26}. The urea/hydrocarbon adducts were prepared by the addition of the calculated amount of hydrocarbon to a saturated solution of urea in either methanol or in *iso*propanol. The solution was cooled slowly in a lagged Dewar vessel. The largest crystals (about 15 mm. long) were removed and dried. The proton magnetic resonance spectra were measured at 25° C. on a Varian 4300 *B* dual-purpose nuclear magnetic resonance spectrometer with a 12-in. magnet. The spectra were observed at a frequency of 40 Mc./s. and a magnetic field of 9,400 gauss.

All the adducts studied showed similar spectra. These showed a broad resonance line of 6·9–7·1 gauss half-width, mainly due to the protons of the urea cage, and a narrow line of about modulation width due to the protons of the enclathrated hydrocarbon molecules. By using minimum modulation (30 milligauss) and sweeping slowly through resonance (0·09 gauss/min.) the narrow resonance line observed for the urea/hexacosane adduct was estimated to be about 0·25 gauss wide. Fig. 1 shows a smoothed outline of a typical proton magnetic resonance spectrum observed for the hexacosane/urea adduct. Fig. 2 shows the proton magnetic resonance spectrum of hexacosane (m.p. 56° C.) itself under the same conditions. The main resonance peak is approximately 8·0 gauss wide, and the second peak is pre-

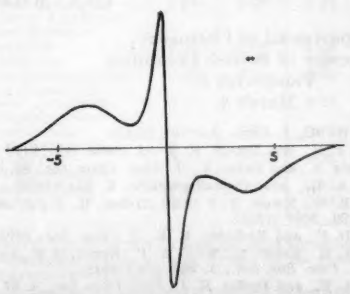

Fig. 1. Smoothed outline of derivative of typical proton magnetic resonance spectrum in *n*-hexacosane/urea adduct at 25° C.

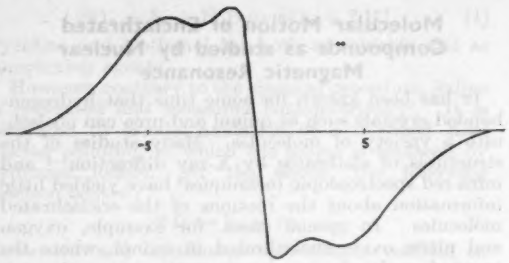

Fig. 2. Smoothed outline of derivative of typical proton magnetic resonance spectrum of *n*-hexacosane at 25° C.

sumably due to rotation of the terminal methyl groups.

To confirm that the narrow resonance line observed (Fig. 1) for the hydrocarbon/urea adduct was due to the protons of the enclathrated hydrocarbon we studied the proton magnetic resonance spectrum of the *n*-hexadecane/urea-d_4 adduct. Only a narrow line comparable with that shown in Fig. 1 was observed. This, of course, indicates that the enclathrated hydrocarbon molecules are rotating with some freedom, or executing torsional oscillations, about an axis parallel to the length of the channel in the urea complex.

In contrast to the results given above for urea/paraffin adducts we have observed that the thiourea/*cyclo*hexane clathrate spectrum at 25° C. has a line width of about 1·4 gauss due to the protons of the *cyclo*hexane molecule. *Cyclo*hexane[9,10] itself has, of course, a very narrow proton magnetic resonance absorption peak at 25° C. The broadening we observed when the *cyclo*hexane is enclathrated in thiourea is obviously due to the restriction of the motion of the *cyclo*hexane molecules in the thiourea cage. The crystal structure[4] of the thiourea/*cyclo*hexane clathrate is known. From this it is easy to see how the normal rotations of the *cyclo*hexane molecule at 25° C. are greatly restricted, or eliminated, when this molecule is enclathrated in thiourea.

We are continuing these researches and are determining the line-widths of the proton magnetic resonance signals over a wide range of temperatures. It is hoped that the results will provide useful information concerning the interaction between the matrix and the enclathrated molecules.

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BIOCHEMISTRY

Milk as an Eluant of Polycyclic Aromatic Hydrocarbons added to Wax

Proteins in aqueous solution have been demonstrated to elute polycyclic aromatic hydrocarbons from soot particles. The liberated hydrocarbons, although hydrophobic, are retained in solution by the proteins[1]. We wished to determine whether milk, a protein solution, would elute incorporated polycyclic aromatic hydrocarbons from paraffin waxes despite the hydrophobic nature of the hydrocarbons.

Dairy waxes as used commercially to impregnate milk cartons were selected as the vehicle for incorporation of polycyclic aromatic hydrocarbons. It should be emphasized that the elution results reported here relate exclusively to the polycyclic aromatic hydrocarbons which were *added*, rather than to those occurring naturally in dairy wax. However, an analysis of several dairy waxes revealed the presence of a small amount of 1,2,5,6-dibenzanthracene of the order of one part per million in one of them. No 3,4-benzpyrene could be detected at levels as low as 1 in 10^9. The two polycyclic aromatic hydrocarbons chosen for elution studies were 3,4-benzpyrene and 1,2,5,6-dibenzanthracene. Both belong to the group of polycyclic aromatic hydrocarbons usually associated with petroleum derivatives, and their presence in unrefined paraffin waxes from which dairy wax is made is probable. Both these hydrocarbons are potent carcinogenic agents. Our interest in 3,4-benzpyrene is related to its ubiquitous environmental distribution; 1,2,5,6-dibenzanthracene is pertinent because of its demonstration in a particular wax used for coating milk cartons.

Table 1. Elution of Added Carbon-14–3,4-Benzpyrene from Dairy Wax (54 hr.)

	Temperature	
	20° C.	5° C.
Whole milk	94 per cent	63 per cent
Half and half*	91 ,,	43 ,,
Buttermilk	81 ,,	48 ,,
Skim milk	74 ,,	43 ,,

* Equal amounts of milk and dairy cream.

Waxed surfaces containing added amounts of both polycyclic aromatic hydrocarbons were prepared in test-tubes so that a surface area of 26 sq. cm., sufficient to hold 10 ml. of milk, resulted. This represents between 3 and 4·6 times the ratio of waxed surface to milk content found in commercially available milk cartons. The thickness of the waxed layer lining the tubes varied from 75 to 250μ, while in milk cartons thicknesses were 75–100μ. The waxed layers were removed from the tubes by freezing in solid carbon dioxide with subsequent thawing. A stereomicroscope was used for measurement. As anticipated, elution from thin layers regularly occurred more rapidly than from thicker layers.

Four types of milk were selected for study: homogenized milk, skim milk, buttermilk, and a milk-cream mixture obtained commercially as 'half and half'. Differences in fat content and *p*H in the four milks chosen were expected to yield clues relative to the mechanisms of elution.

Room temperature and usual refrigerator temperature (5° C.) were selected for comparative studies as these are the two commonly encountered in milk

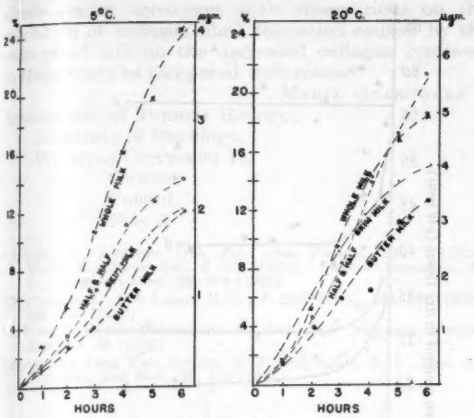

Fig. 1. Elution by milk of 3,4-benzpyrene added to wax

use. In numerous experiments we established that the elution at the two contrasting temperatures of 20° C. (room temperature) and 5° C. (refrigerator temperature) for the various milks was consistent and compatible. We therefore abandoned attempts at attaining identical thickness of wax and concentration of carcinogen when consonant results had been obtained.

The quantity of benzpyrene eluted by milk was determined at hourly intervals for the first 6 hr. of exposure by the difference in intensity of fluorescence between the maximum at 405 mμ and the minimum at 427 mμ. The increase in fluorescence intensity is given in Fig. 1 for each of the milks at room temperature and at 5° C. As we wished to obtain additional information on the completion of elution, the exposure of wax to the particular milks was continued for 24 hr. After the 24-hr. exposure, individual waxes were exposed to fresh samples of each type of milk for an additional 30 hr. At this time, fluorescence could be demonstrated primarily in the upper rim of wax which had not been in contact with the milk. At one extreme, for example, the total amount of benzpyrene retained in the wax after 54-hr. contact with a butter-milk sample was found to be 1·8 per cent. Carbon-14–1,2,5,6-dibenzanthracene was used rather than the unlabelled pure crystalline compound, as the minute amounts of the hydrocarbon used did not possess enough fluorescence energy to be detected.

Elution of benzpyrene and dibenzanthracene was observed in all milk samples studied. Homogenized milk was the best eluant, with the remaining three showing slightly less potency. There was almost total elution of polycyclic aromatic hydrocarbons during a 54-hr. period. The decrease in rate of elution incidental to the depletion of the waxes occurred as expected. At room temperature, the elution was more complete for all four types of samples.

The presence of the polycyclic aromatic hydrocarbons in milk cannot be attributed to a possible solubilization of the wax in milk. This was established by the addition of a strongly fluorescent wax to milk in all experimental circumstances and the subsequent failure to demonstrate fluorescence in the milk.

Milk, independent of its fat content, is an efficient eluting agent and solvent of benzpyrene and 1,2,5,6-dibenzanthracene from hydrophobic wax following the incorporation of these carcinogenic hydrocarbons into wax. This occurs in a manner that is compatible with both the temperatures usually encountered and the duration of contact of milk with the waxed carton.

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Detection of Hydroxyl Groups in Porphyrins and Chlorins

The behaviour of porphyrin a [1] and chlorin a_2 [2], the iron-free prosthetic groups of cytochrome a and a_3 and cytochrome a_2 respectively, when run as the methyl esters on paper chromatograms in various solvent systems, suggests that these compounds contain a strongly polar group in one of the side-chains. In the case of porphyrin a, the formyl group, which is known to be present, would not alone account for the R_F values obtained.

To test the possibility that the polar characteristics of these two tetrapyrroles were due to the presence of a hydroxyl group in the molecule, the following technique was devised. The porphyrin, or chlorin, was acetylated in a mixture of acetic anhydride and pyridine (1 : 10 v/v) at room temperature overnight, the mixture was then poured into ether and the ether washed well with 0·1 per cent hydrochloric acid followed by distilled water. Paper chromato-

Table 1. R_F Values of Hydroxylated Porphyrins and Chlorins, and their Acetylated Products

Compound (methyl esters)	Chloroform–kerosene		Propanol–kerosene	
	Alcohol	Acetate	Alcohol	Acetate
1. Monohydroxyethyl deuteroporphyrin	0·34	0·64	0·38	0·68
2. Monovinyl-monohydroxyethyl deuteroporphyrin	0·29	0·60	0·34	0·66
3. Hæmatoporphyrin	0·03	0·56	0·18	0·57
4. Monohydroxymethyl deuteroporphyrin	0·19	0·54	0·24	0·57
5. Monohydroxymethyl monovinyl deuteroporphyrin	0·22	0·54	0·31	0·56
6. Dihydroxymethyl deuteroporphyrin	0·01	0·56	0·14	0·56
7. 2-Formyl-4-hydroxyethyl deuteroporphyrin	0·16	0·58	0·26	0·64
8. Porphyrin a	0·10	0·56	0·26	0·62
9. Dioxyprotoporphyrin*	0·15	0·56	0·30	0·58
10. 2-Ethyleneglycol deuteroporphyrin	0·30	0·64	0·32	0·61
11. 2-α-Hydroxymesorhodochlorin	0·31	0·80	0·34	0·78
12. 2-α-Hydroxymesochlorin p_4	0·33	0·82	0·35	0·80
13. 2-α-Hydroxymesophaephorbide a	0·10	0·78	0·34	0·72
14. Chlorin a_2	0·30	0·65	0·40	0·60

Compounds 3, 7, 8, 9, 13 and 14 were prepared according to known methods, compounds 1, 2, 4, 5, 6, 10, 11 and 12 by methods which will be published elsewhere in detail.

* This compound contains only one hydroxyl group and one acetyl side-chain (to be published elsewhere).

graphy of the acetates was then carried out in the chloroform–kerosene, or the propanol–kerosene systems of Chu *et al.*[3], and the R_F values obtained compared with those of the parent compound. These R_F values and those of a number of model hydroxylated porphyrins and chlorins and their acetates are given in Table 1.

The difference between the R_F value of a compound with a free hydroxyl group and that of its acetate is of some value in distinguishing between the presence of one or more hydroxylated side-chains in the molecule.

From the values given in Table 1 it is clear that porphyrin *a*, chlorin a_2, and dioxyprotoporphyrin[4], an irradiation product of protoporphyrin, each contain a hydroxylated side-chain, probably only one in each compound.

Further work is being carried out in this laboratory to determine the nature and position of the hydroxyl groups in these tetrapyrroles.

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Collagen Content of Normal Connective Tissue, of Tissue surrounding a Tumour and of growing Rat Sarcoma

There have been several studies of the qualitative and quantitative changes produced in different components of connective tissue after treatment of normal animals with carcinogens[1]. However, little attention has been paid to the changes in the connective tissue during the growth of a tumour. This communication reports studies in the collagen content of neoplastic tissue, as well as of the tissue surrounding the tumour, during the development of the neoplasm in rats. The quantities of collagen in subcutaneous tissue taken from the upper part of the abdomen (at a considerable distance from the tumour) were taken as controls.

The experiment was carried out on male and female white rats aged 2–4 months. A fragment of Guérin carcinoma was implanted subcutaneously into the backs of 11 male rats, which formed one group of animals, and a fragment of transplantable sarcoma (42nd passage) first induced by methyl cholanthrene was implanted into the second group of 30 rats comprising both males and females. At different intervals between 3 and 30 days after grafting the tumour the animals were killed, and specimens of the tissues mentioned above were taken for determination of collagen by the Neuman–Logan method[2]. The method was modified by shaking the sample with the same volume of ethyl acetate just before reading the absorption. In this way the coloured impurities originating in either the reagent or the extract were removed. Fig. 1 shows the collagen

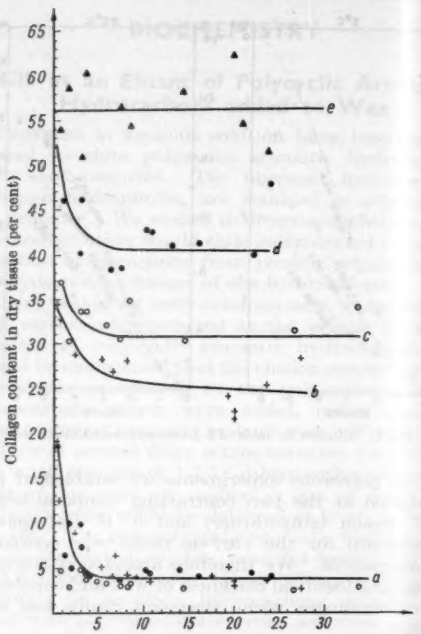

Fig. 1. *a*, Collagen content in rat neoplastic tissue during development of Guérin carcinoma in males (○) and during growth of sarcoma in males (●) and females (+). *b*, *c*, *d*, Collagen level in the tissue surrounding tumours under investigation. *e*, Arithmetic mean of collagen determinations in normal subcutaneous tissue of male tumour hosts (▲)

content of the tissues investigated. Every point represents the mean of three parallel analyses.

Curve *a* shows the dependence of collagen content in the neoplastic tissue on weight of tumour. In the first stage of development of the tumour there is a rapid decrease in the collagen content, after which its level remains unchanged. The levels of collagen in neoplastic tissue of male and female rats bearing sarcoma as well as of males bearing Guérin carcinoma are of the same order. There are no significant differences in the collagen content depending either on the sex of the host or on the kind of tumour.

Curves *b*, *c* and *d* represent determinations of collagen in tissue surrounding the tumour. In contrast to the neoplastic tissue, here the level of collagen depends significantly on the sex of the host. The collagen contents in males bearing sarcoma are almost twice as high as in females bearing the same tumour. Moreover, in males the tissue surrounding the carcinoma contains less collagen than that surrounding the sarcoma.

The line, *e* at the top of Fig. 1 represents the arithmetic mean of the collagen determinations; it characterizes the quantity of collagen in the normal subcutaneous tissue of male tumour hosts. It is evident that the collagen-level in normal tissue is higher than that in the tissue surrounding the tumour. This suggests that the subcutaneous tissue reacts to the tumour either by decreased synthesis or by increased catabolism of collagen.

The significant difference in the level of collagen in tissue surrounding the tumour in male and female rats bearing the sarcoma probably is the result of hormonal differences. The findings described

above are in agreement with observations on the inhibition of collagen fibril formation caused by the œstrogens[2] and on the decreased collagen synthesis in female rats as compared with males[4].

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Glutamic–Aspartic Transaminase of Pig Heart Muscle

THE glutamic–aspartic transaminase of pig heart muscle has been more than 98 per cent resolved into apoenzyme and co-factor by carrying out a differential heat inactivation in phosphate buffer in the course of purification of the enzyme. The apoenzyme has been purified 64-fold, by relatively simple and reproducible techniques. The highest purification factor previously reported[1] for the apoenzyme is 26. The specific activity of the purified apoenzyme compares favourably with that of an electrophoretically homogeneous preparation of the holoenzyme[2]. Cammarata and Cohen's suggestion[3] that purification of the apoenzyme necessarily results in partial inactivation has been disproved, since it is possible to account for all the enzyme activity among the various protein fractions obtained at each step in the purification.

The initial rate of reaction between aspartate and α-ketoglutarate is linearly dependent on the concentration of the purified transaminase only up to 0·004 mgm. protein c.c.$^{-1}$. Above this level the dependence of reaction-rate on enzyme concentration deviates markedly from a linear relation. For the reverse reaction, the dependence of the reaction-rate on enzyme concentration is linear up to 0·01 mgm. protein c.c.$^{-1}$. This phenomenon has been reported for crude enzyme preparations[3,4]; but its consequences have not been considered in any kinetic studies reported hitherto. The following evidence does not support the suggestion[4] that the non-linear dependence of reaction-rate on enzyme concentration is due to the existence in solution of an equilibrium between active and inactive forms of the enzyme. First, the initial rate of reaction is unaffected by reagents known to break down protein aggregates, for example, benzene, ionic detergents and high salt concentrations. Second, when the same low concentration of pyridoxal-5′-phosphate (4γ c.c.$^{-1}$) is used for reactivation of a concentrated and dilute solution of the apoenzyme, equivalent amounts of enzyme taken from the two preincubation mixtures give the same initial rate of transamination on addition to the substrates. This shows that if aggregates are formed at high protein concentrations the aggregation is such that the sites occupied by the co-factor are not masked. Third, the proposed equilibrium between active and inactive forms of the enzyme must be established very rapidly since the observed initial rates of reaction depend only on the enzyme concentration in the reaction mixture and not on the concentration of the enzyme during the period of preincubation with the co-factor.

The variation of the rate of reaction with enzyme concentration is independent of the purity of the enzyme and is unaffected by the addition of a boiled extract of pig heart muscle. It is therefore unlikely that the non-linear dependence of reaction-rate on enzyme concentration is due to the presence of an inhibitor in the original protein extract.

Bonavita and Scardi[5] concluded that the co-factor was attached to the enzyme through the 4-formyl group and that this group was not free to take part in Schiff's base formation with the substrates as suggested by Snell and co-workers[6]. This conclusion was based on the absence of inhibition of transamination by cyanide ions when added, in excess, to the reactivated enzyme followed by 12 hr. dialysis before assay, whereas inhibition did occur when the cyanide ions were preincubated with the co-factor before reactivation of the apoenzyme. In the present work, using cyanide ions and pyridoxal-5′-phosphate in equimolar concentrations, the same degree of inhibition (75 per cent) was observed both when the cyanide ions were added to the reactivated enzyme and when the co-factor was incubated with cyanide prior to reactivation of the apoenzyme.

The equilibrium constants for the reaction between the apoenzyme and the two forms of the co-factor have been measured under conditions in which there can be no shift in the equilibrium during the assay of the holoenzyme concentration. Previous determinations of the equilibrium constants[1,7] have been unsatisfactory because such precautions have not been taken. It appears that the true equilibrium constant for pyridoxal-5′-phosphate is some ten times greater than that for pyridoxamine-5′-phosphate.

Braunstein[8] has suggested that the equilibrium constant for the overall transamination reaction depends on the purity of the transaminase preparation used. The equilibrium constant has been determined using a highly purified enzyme solution, and the mean value of K, from some twenty determinations, is 6·50, in close agreement with the value of 6·74 found by Krebs[9] using a crude extract of horse heart muscle.

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Deoxyribonucleic Acid Content of the Cell-Nuclei in the Adrenal Medulla after Exposure to Low Temperatures

As has been shown by H. Roels[1] in other endocrine glands, *I have found that, in the adrenal medulla of* the white rat, there is a correlation between the mean deoxyribonucleic acid content of the nuclei and cell activity. A preliminary communication on this topic[2] reports an increase of the nuclear deoxyribonucleic acid content after stimulation by means of daily injections of insulin, and also an important decrease in the resting gland after bilateral splanchnectomy.

The present communication is devoted to experiments on stimulation of the adrenal medulla by means of exposure to low temperature; the stress produces hypersecretion of adrenaline.

Five male rats were kept for a fortnight in a refrigerator at nearly 4° C. They were killed, together with five controls. The adrenals and a control testis were simultaneously fixed in alcohol, formol and acetic acid (75 : 20 : 5), dehydrated and embedded. Sections (10μ) of the different objects were mounted on the same slide, in order to obtain homogeneous Feulgen staining. The deoxyribonucleic acid content of the individual nuclei was measured *in situ* by Lison's histophotometrical method[3]. As the deoxyribonucleic acid contents obtained were given in arbitrary units, we used as a standard a 'theoretical diploid value', being half the mean content of 50 spermatocytes I measured in the control testis. The results are summarized in Table 1.

Table 1

		$\bar{x}$	$\log \bar{x}$	s	$s \log \bar{x}$	n
Spermatocytes I		2,663	3·425	0·004	0·006	50
			Theoretical 2s value, 1,332			
Controls	284	1,308	3·116	0·046	0·006	50
	285	1,366	3·135	0·067	0·009	50
	286	1,200	3·079	0·045	0·006	50
	287	1,210	3·082	0·055	0·007	50
	288	1,267	3·102	0·053	0·007	50
	Pool	1,268	3·103	0·052	0·003	250
Cold-treated	289	1,469	3·167	0·043	0·006	50
	290	1,426	3·154	0·039	0·005	50
	291	1,389	3·142	0·042	0·005	50
	292	1,372	3·136	0·034	0·004	50
	293	1,446	3·160	0·039	0·005	50
	Pool	1,414	3·150	0·041	0·008	250

As the different individual deoxyribonucleic acid contents of the nuclei present a log-normal distribution, the statistical computations are made using the logarithms of these values.

Comparing cold-treated animals and controls, it is clear that the stimulation of the adrenal medulla is accompanied by an increase in the mean deoxyribonucleic acid content of the nuclei, which for the pooled results reaches 11·4 per cent ($t = 7·0$; $P < 0·001$).

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New Types of Salting-out Paper Chromatograms of Antibiotics

According to Miyazaki, Omachi and Kamata[1], antibiotics can be grouped according to their salting-out chromatograms. Pure antibiotics and materials containing antibiotics were examined by the aid of ascending paper chromatography. As irrigation solvents in each case and with each antibiotic, distilled water and rising concentrations of ammonium chloride (0·5, 1, 2, 3, 5, 10, 20 per cent and saturated) were used. Location of the spots of the antibiotics, that is, the R_F values, were ascertained bio-autographically.

Antibiotics were divided into four groups. In those of group *A* the R_F value is not correlated with the concentration of the ammonium chloride solution, identical values being found at all concentrations and in distilled water. To this group belong penicillin, grisein, chloramphenicol, aureothricin, and the special substance 2. In group *B* are those antibiotics which have an R_F value 0 in distilled water, and increasing R_F values with increasing concentrations of ammonium chloride. They are: streptomycin, streptothrycin, luteomycin, fuscomycin, xanthomycin and flaveolin. Group *C* is different from group *B*, displaying the highest R_F value in distilled water, and lowering of the values with increasing concentration of the solution of ammonium chloride. Members of this group are: actinomycin and griseoflavin. Group *D* consists of antibiotics which do not display any movement whatever in an irrigating solvent from the starting point; eventually increased tailing tendency was observed with higher concentrations of the solutions of ammonium chloride. Aureomycin and terramycin are members of this group.

These results obtained by Miyazaki *et al.* have been confirmed by my experiments with salting-out paper chromatography of antibiotics. Moreover, I have been able to show the existence of other types of salting-out paper chromatograms of antibiotics with antibacterial action, of actinomycetal origin.

In group *E* are antibiotics which have an R_F value 0 in distilled water, and an initial increase with rising concentrations of the solution of ammonium chloride, the maximum being (R_F = nearly 1) in 5 per cent ammonium chloride. Thereafter a decrease occurs, as shown in Fig. 1. The line connecting the different R_F values is of the shape of a truncated parabola. This type of paper chromatogram was

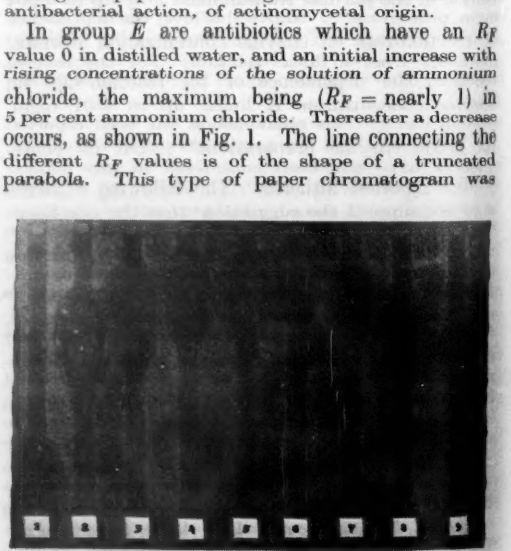

Fig. 1. The salting-out paper chromatogram of erythromycin. 1, Distilled water; 2, 0·5; 3, 1; 4, 2; 5, 3; 6, 5; 7, 10; 8, 20 per cent; and 9, saturated ammonium chloride solutions

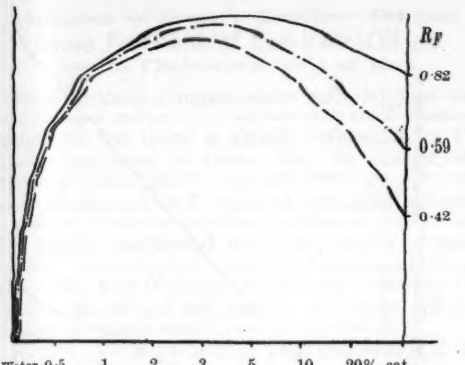

Fig. 2. Curves of the salting-out paper chromatograms of three macrolides. ——, Oleandomycin; ·—·—·, erythromycin; – – – – –, carbomycin

found in the well-known macrolides (for example, oleandomycin, erythromycin and carbomycin). Within the group, further differentiation of these three macrolides was possible. The curvature of the truncated parabola—best demonstrated by the R_F value in saturated solution of ammonium chloride—is different in these three compounds. The slightest curvature occurred with oleandomycin, the R_F value in saturated ammonium chloride being 0·82; the maximal one with carbomycin (under identical conditions the R_F was 0·42). A midway position is occupied by erythromycin (R_F value being 0·59), as shown in Fig. 2. Obviously, the curvature of the truncated parabola is proportional to the molecular weight of these three macrolides. With the least curvature for oleandomycin is associated the least molecular weight (689); with the greatest curvature of carbomycin the highest one (844). As regards molecular weights, erythromycin is again midway between these (733).

At present, only one antibiotic can be placed in group *F*. This is desertomycin, produced recently by Úri *et al.*[2]. The characteristic feature of the salting-out paper chromatogram is the 0 value of R_F in distilled water. A rise of the R_F values is associated with increasing concentration of ammonium chloride up to 3–5 per cent; thereafter, however, R_F values decrease, and the value $R_F = 0$ is associated with saturated ammonium chloride solution. This type of chromatogram is shown in Fig. 3. By connecting the single R_F values, a paraboloid curve is obtained. This seems to unite groups *B* and *C* of Miyazaki *et al.*, the main representatives being streptomycin with primarily antibacterial activity, and actinomycin which is endowed with a cytostatic effect also. Desertomycin exerts an antibacterial as well as a cytostatic activity, as I have shown. Since this type of salting-out paper chromatogram has not been reported among antibiotics hitherto studied, in addition to its other characteristics, this feature is further proof that desertomycin is a new antibiotic.

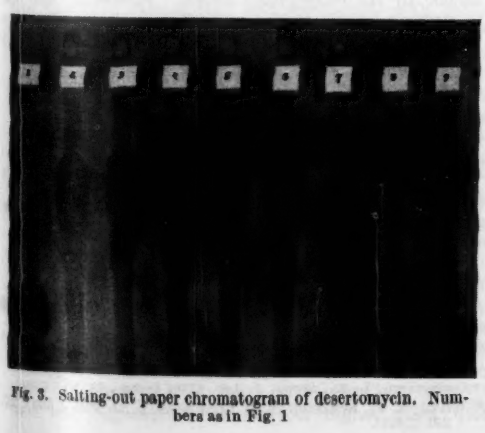

Fig. 3. Salting-out paper chromatogram of desertomycin. Numbers as in Fig. 1

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ANIMAL PHYSIOLOGY

Thyroxine-releasing Effect of Salicylate and of 2,4-Dinitrophenol

THE fact that salicylate increases the oxygen consumption in man was first observed by Denis and Means[1] in 1916. This observation has since been corroborated by several other investigators[2]. The oxygen consumption of tissues removed from animals treated with salicylate is likewise increased[3].

Coincident with the increase in metabolic rate, there is a decrease in the protein-bound iodine of the serum, and the fractional rate of disappearance of injected radiothyroxine from the circulation increases[2]. Furthermore, salicylate causes a reduction in the uptake of radioiodine by the thyroid[3], but the uptake is increased after stimulation by thyrotropin[4]. Similarly, 2,4-dinitrophenol can induce a rise in basal metabolic rate and a coincident fall in protein-bound iodine in the serum[5]. In experiments with laboratory animals, it has been found that dinitrophenol may reduce the uptake of iodine-131 by the thyroid[6]. The mechanisms by which salicylate and dinitrophenol act have not been elucidated.

All these observations could, however, be explained by an increase in the plasma level of free thyroxine, that is, thyroxine not bound to protein. It is presumably the concentration of free thyroxine which controls the quantitative biological effect of the thyroid hormone[7].

Austen, Rubini, Meroney and Wolff[2] found no change in the concentration of thyroxine-binding protein after salicylate administration. They concluded that the effects of salicylate could not be due to a change in the binding of thyroxine.

I have recently described a method for direct determination of free plasma thyroxine[8] which has permitted a renewed investigation of this problem.

The interaction between thyroxine (*Tx*) and thyroxine-binding protein (*P*) may be expressed by the reaction scheme:

$$Tx + P \rightleftarrows TxP$$

where the equilibrium is displaced so far to the right that less than one-thousandth of the total thyroxine is free.

Application of the law of mass action gives:

$$[Tx_f] = k\,\frac{[PBI]}{[P_{unsat.}]} \qquad (1)$$

where Tx_f denotes free thyroxine and $P_{unsat.}$ unsaturated thyroxine-binding protein. On the assumption that all organic iodine present is in the form of thyroxine, TxP has been replaced by PBI in the equation.

It should thus be possible to obtain an estimate of $[Tx_f]$ if the unsaturated protein could be determined in addition to the protein-bound iodine, which may be analysed chemically.

If a tracer dose of radiothyroxine ($Tx^{131}I$) is added to a sample of serum, the following relationship will be valid after equilibrium has been established:

$$\frac{[Tx^{131}I_f]}{[Tx_f]} = \frac{[PB^{131}I]}{[PBI]} \qquad (2)$$

When the expression (1) for $[Tx_f]$ is inserted in this equation, we obtain:

$$\frac{[Tx^{131}I_f]}{[PB^{131}I]} = \frac{K}{[P_{unsat.}]}$$

Thus it is seen that the denominator of the fraction in equation 1 can be calculated by determining the fraction of the added quantity of radiothyroxine that is present in the free state. ($[Tx_f]$ can also be calculated directly from equation 2.)

The determination was carried out as follows. A 'Plexiglas' vessel consisting of two separate, identical parts was assembled with a 'Cellophane' membrane between the two halves. In one of the chambers thus formed was placed 10 ml. of serum, to which radiothyroxine was added (chamber A). In the other chamber was placed 10 ml. of serum to which the same quantity of normal thyroxine was added (chamber B). The assembly was incubated in a constant-temperature bath with adequate stirring of the solution containing the radiothyroxine.

The free $Tx^{131}I$ passes through the membrane at a rate proportional to its concentration. During the first 24 hr., two samples of 4 ml. or three of 3 ml. were withdrawn from chamber B. At the same time an equal volume was removed from chamber A.

Since the $Tx^{131}I$ employed contained some inorganic iodine-131, the samples had to be washed carefully[9], after which the content of $Tx^{131}I$ was determined in a well-type scintillation counter. The result is expressed as per cent of the total $PB^{131}I$ in chamber A. Details of the experimental procedure are described elsewhere[6].

The results are shown in Fig. 1 and in Table 1.

Table 1. Effect of Addition of 2,4-Dinitrophenol

2,4-Dinitrophenol added (mgm./100 ml.)	Percentage increase of free thyroxine
0·5	9
1·0	18
3·0	25
5·0	38

As can be seen from Fig. 1 the salicylate evidently causes a substantial increase in the amount of $Tx^{131}I$ passing through the membrane. According to the above considerations this must be due to an increase in free thyroxine.

The thyroxine-releasing effect of salicylate and of dinitrophenol is probably a result of binding of these substances to some of the thyroxine-binding sites of the plasma proteins. Whether this is true only of the albumin or whether it also applies to the specific

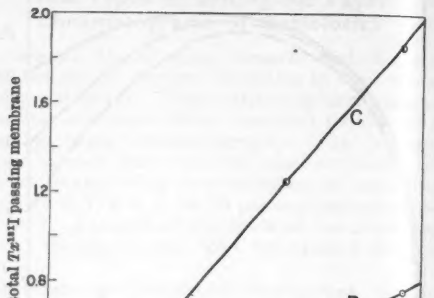

Fig. 1. *A*, Amount of $Tx^{131}I$ passing through the membrane at various times; *B*, effect of 10 mgm. sodium salicylate per 100 ml. added to both chambers; *C*, as *B* with 50 mgm. sodium salicylate

thyroxine-binding protein cannot be decided on the basis of the present investigations.

After administration of an ordinary dose of salicylate, the level in the plasma is in the range[2] 20–50 mgm./100 ml. Thus the above experiments can explain the rapid calorigenic action of salicylate, and also the fact that the action is transient. The increase in free thyroxine explains the increased fractional rate of disappearance of injected radiothyroxine and the fall in PBI. Furthermore, the fall in the uptake of radio-iodine by the thyroid, and the increase after administration of thyrotropic hormone, may be explained by the increase in free thyroxine.

Thus, the experiments definitely support the idea that it is the free plasma thyroxine that regulates the pituitary effect on the thyroid.

The dose of dinitrophenol that causes a calorigenic effect in man is 200–300 mgm./24 hr. period. This dose may well give rise to plasma concentrations of dinitrophenol within the concentration-range in Table 1. Possibly dinitrophenol also has a direct effect on the uncoupling of oxidative phosphorylation[10]. The effect may even be a more important factor in its calorigenic effect. However, the fall in protein-bound iodine and the possible fall in the thyroid uptake of radioiodine can presumably only be explained by the increase in free thyroxine.

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Influence of Dietary Cod-liver Oil and Some Fractions of Cod-liver Oil on Serum Cholesterol-level of Rats

FROM numerous experiments with humans and experimental animals it is known that the cholesterol content of the blood is greatly influenced by the amount and kind of dietary fat. In general, the relatively unsaturated vegetable fats decrease the serum cholesterol-level, while an increasing influence is observed with the relatively saturated animal fats. The highly unsaturated oils from marine animals, however, exert a potent cholesterol-lowering activity. This effect was observed in humans following the consumption of seal oil[1], sardine oil[2], whale oil[3] and pilchard oil[1,4] and also in rats fed sardine oil[5].

So far it is not yet decided what compounds of the fats are responsible for the action on cholesterol. The polyenoic acids, linoleic acid and arachidonic acid, applied in pure form, were found to be very active in lowering serum cholesterol-levels[6], whereas hydrogenation of highly unsaturated fats was accompanied by a decrease in activity[3,7]. These findings suggest that the fatty acid fraction is responsible for the effect observed. However, other experiments suggest that the unsaponifiable fraction of vegetable oils possesses cholesterol-lowering properties[8,9].

We have studied the cholesterolemic properties of cod-liver oil and some fractions thereof by feeding them to groups of 10–12 newly weaned rats in a hypercholesterolemic basal diet of the following percentage composition: casein 15, potato starch 10, wheat starch 57·5, minerals 4, vitamin mixture 2·1, choline chloride 0·2, hydrogenated coconut oil 10, cholesterol 1·0 and cholic acid 0·2. Different fats or fractions of cod-liver oil were added at the expense of equal amounts of wheat starch. Fractions of cod liver oil were prepared after saponification with potassium hydroxide in the presence of ethanol. The soap was split by the addition of hydrochloric acid and the resulting fatty acids washed and dried in a centrifugal separator. The fatty acids were then allowed to crystallize for 24 hr. at 15° C. and filtered through a bag filter at the same temperature. The fatty acid fractions were esterified with ethyl alcohol in the presence of sulphuric acid and the resulting esters refined, washed and dried under vacuum. The unsaponifiable matter was isolated by extracting a diluted solution of cod-liver oil soap with methylated ether. After three extractions, the solvent was removed from the washed extract by evaporation, the residue re-saponified and extracted with ether. The ethereal extract was then washed with dilute potassium hydroxide solution, followed by water washes until neutral, evaporated and dried under vacuum. The aqueous soap solution from which the unsaponified matter had been extracted was freed from dissolved ether, the soap split with acid and the fatty acids purified and esterified as above to give the fatty ester fraction free of unsaponifiable matter.

After feeding the experimental diets *ad lib.* over four weeks, the amount of total serum cholesterol of each rat was determined by the Liebermann–Burchard reaction.

The results presented (Table 1) show that the growth-rate was very poor, that is, only half that obtained on a stock ration. This may be ascribed to the sub-optimal protein content and the high fat content of the diet and to the presence of cholesterol and cholic acid. With corn oil in the diet, growth-rate was highest and somewhat higher than with cod-liver oil. The esterified fatty acid fractions caused a decrease in food intake which was paralleled by a slower growth-rate.

Table 1. MEAN GAIN IN WEIGHT, FOOD CONSUMPTION AND SERUM CHOLESTEROL

Addition to basal diet	Per cent	Daily weight increase (gm.)	Daily food consumption (gm.)	Total serum cholesterol (mgm./100 ml.)
Hydrogenated coconut oil	10	1·6	6·5	784
Corn oil	10	2·3	7·2	430
Cod-liver oil	10	2·1	7·0	196
Ethyl esters of fatty acids liquid at 2° C.; iodine value 189	10	1·6	5·9	241
Ethyl esters of fatty acids liquid at 15° C.; iodine value 161	10	1·7	6·3	240
Ethyl esters of fatty acids solid at 15° C.; iodine value 135	10	1·7	6·3	277
None		2·0	8·8	884
Cod-liver oil	10	1·7	6·6	205
Unsaponified matter	0·4	2·0	8·0	750
Ethyl esters of fatty acids	10	1·2	5·9	281
Unsaponified matter + ethyl esters of fatty acids	10	1·5	6·2	321

The serum cholesterol-level obtained with corn oil was much lower than with hydrogenated coconut oil. With cod-liver oil a significantly lower value was obtained than with corn oil ($P = 0{\cdot}003$ according to Wilcoxon's test). The ethyl esters of cod-liver oil fatty acids decreased the cholesterol content to nearly the same degree as did whole cod-liver oil. The mean serum cholesterol values obtained with the cod-liver oil fractions with different melting points were not significantly different. This may be the result of incomplete separation or of equal cholesterol-lowering properties of the various fatty acids present in the different fractions. The unsaponifiable fraction, in an amount equivalent to the level present in the whole cod-liver oil, exerted no distinct hypocholesterolemic action. The combination of unsaponified fraction with the esterified fatty acid fraction resulted in a mean value not significantly different from that obtained with whole cod-liver oil ($P > 0{\cdot}05$).

From these results it is concluded that cod-liver oil has a higher cholesterol-lowering activity than corn oil. The fatty acid fraction accounts for most if not all of the activity. The unsaponifiable fraction shows no distinct activity.

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Serological Relationships in the Genus *Gadus*

Little information is available concerning the serological relationships of fishes, and virtually nothing is known concerning the serology of the economically important genus *Gadus*.

Three species from this genus have been investigated in our laboratory, namely, the cod (*Gadus callarias* L.), the haddock (*G. aeglifinus* L.) and the pollack (*G. pollacius* L.). Blood was obtained from these three species by opening the animal after stunning, and taking it directly from the heart. The blood was allowed to clot, the serum separated, and merthiolate added to give a concentration of 1 : 10,000. The stock of rabbits used proved rather refractory, and the potency of the antisera was lower than might have been hoped. Three moderately satisfactory antisera were obtained, the first an anti-haddock serum *R* 71, and two anti-cod sera *R* 72 and *R* 712. Each of these antisera was produced after a course of intravenous injections of the fish serum. In the case of the anti-haddock serum, the intravenous course was followed, after a month's rest, by one series of intraperitoneal injections. In the case of the anti-cod serum, *R* 712, the second series of injections were subcutaneous. Anti-cod serum *R* 72 was produced by a single course of four intravenous injections. The amount used for each injection was 0·25 ml. of serum, and a course consisted of either three or four injections, given on alternate days. The characteristics of the antisera are shown in Table 1.

Table 1. Characteristics of the Antisera

Antiserum	Serum used from :	Reciprocal of homologous titre	Homologous photroner curve area
R 71	Haddock	1,000	107
R 72	Cod	1,000	84·5
R 712	Cod	10,000	92·8

The serological correspondence between the various species was determined using the photronreflectometer as described by Boyden and Delfaco[1]. The relationship ratios are given in Table 2. These ratios are calculated by expressing the area under the curve ($\sigma\tau$ value) of the second of the pair being compared as a percentage of the first. The reciprocal figures are also included (asterisks). Thus the cod/pollack ratios are derived from curves for antisera *R* 712 (100 : 21) and *R* 71 (27 : 100). The curves obtained from antiserum *R* 71 are shown in Fig. 1.

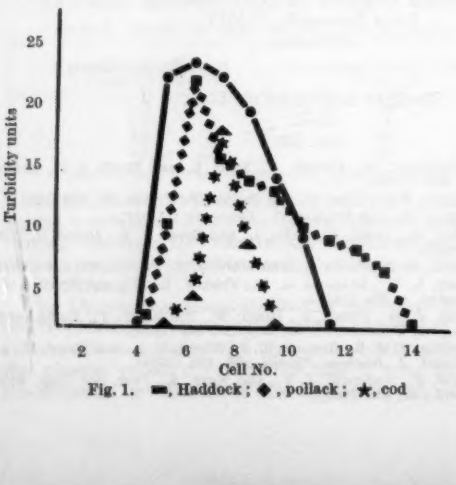

Fig. 1. ▬, Haddock ; ◆, pollack ; ★, cod

Table 2. Relationship Ratios

	Antiserum used	Ratio	Mean
Cod / Haddock	*R* 712	100 : 24	25·3
	R 72	100 : 37	
	R 71	100 : 25*	
Cod / Pollack	*R* 712	100 : 21	24·0
	R 71	100 : 27*	
Haddock / Pollack	*R* 71	100 : 94	90·5
	R 712	100 : 87*	

* Reciprocal figures.

It will be seen that serologically haddock and pollack are closely related and that the cod is about equally remote antigenetically from both.

We are grateful to the Medical Research Council of Ireland for a grant to purchase the photronreflectometer used in these studies and to the Director, and Mr. R. J. Beverton, of the Lowestoft Fisheries Laboratory for sending fish sera and for enabling me to visit the Arctic in the summer of 1956 to collect the cod and haddock sera.

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Cephalotoxin : the Crab-paralysing Agent of the Posterior Salivary Glands of Cephalopods

The presence of a substance, toxic to crabs, in the posterior salivary glands of cephalopods (*Giftdrusen* of the German authors) has been known for a long time[1]. When a drop of saliva collected from *Octopus* is injected into a crab, a sequence of reactions is observed followed by complete paralysis of the animal[2]. Active substances have been extracted from the posterior salivary glands of octopuses[3], and their presence has been demonstrated in external and internal secretions[4]. Most of these substances have a powerful action on crabs and upon isolated organs of molluscs and other invertebrates[5]. Tyramine, the substance first isolated from the glands by Henze[6], was, and still is, believed to be the toxic agent[7]. However, none of the extractive substances found in the posterior salivary glands of cephalopods, even tyramine, can reproduce the paralysing action of the crude extracts or of saliva. The observations of Livon and Briot[8], recently confirmed by me[9], claim a more complex composition. The active principle is inactivated by heating, does not pass through dialysing membranes and can be purified by precipitation with ammonium sulphate.

From the posterior salivary glands of *Sepia officinalis* we have now purified a protein which, when injected into crabs, has the same effect as the dialysed extract from the same organ. The active protein has been called cephalotoxin.

As a measure of purification an arbitrary unit was taken which is defined as the amount of toxin which paralyses in 15 min. a crab weighing 40–50 gm. The animal used in all the experiments was the crustacean decapod *Eriphia spinifrons*. Control animals were injected with equal amounts of extract previously heated at 100° C. for 5 min. Specific activity was calculated as units per mgm. of protein. The protein content was determined colorimetrically with the Folin-phenol reagent[10].

After several attempts the simplest and easiest procedure for extraction and purification of cephalotoxin was found to be the following.

The organs are homogenized in a Waring blendor with 2 vol. cold 0·5 per cent potassium chloride, extracted for 4 hr. in the cold with continuous stirring and centrifuged at 800*g*. The precipitate is suspended again in 1 vol. potassium chloride solution, extracted, centrifuged as before and then discarded. The combined supernatants give: units per ml. 15; protein mgm. per ml. 23·0; specific activity 0·6. The extract is brought to *p*H 4 by careful addition of 2 *M* acetic acid. The precipitate is discarded by centrifugation at 15,000*g*. The supernatant is neutralized with 1 *N* sodium hydroxide and the new precipitate is centrifuged off and discarded. (Units per ml. 15; protein mgm. per ml. 14; specific activity 1·0.) The clear supernatant is dialysed against water for 24 hr. A white precipitate is formed which is discarded by centrifugation. (Units per ml. 15; protein mgm. per ml. 8·0; specific activity 1·9.)

The final extract can be concentrated in the cold or freeze-dried. The powder, redissolved in a small amount of water, gives a positive biuret and ninhydrin reaction and an ultra-violet absorption with a maximum at 276–278 mμ. After electrophoresis on starch gel[11] at *p*H 8·5, four bands are observed migrating towards the cathode. Further purification of the extract can be achieved by adsorption on calcium phosphate gel at neutral *p*H: three components are now visible after electrophoresis. Treatment with trypsin at 37° C. and neutral *p*H produces complete loss of the activity and disappearance of the bands within 3 hr.

Cephalotoxin can be extracted from the posterior salivary glands of *Octopus vulgaris* and *O. macropus* by a procedure similar to that described for *Sepia*.

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PLANT PHYSIOLOGY

Effect of Heavy Water on the Growth of *Chlorella vulgaris*

Several investigators have studied the effect of heavy water on the growth of algae[1–3]. All workers find that heavy water inhibits growth and, in concentrations approaching 100 per cent, no growth occurs. We have found recently that, while autotrophic growth of *Chlorella* is inhibited completely in 90 per cent (v/v) heavy water, growth on glucose, either in light or in darkness, is inhibited to a much smaller extent.

Chlorella vulgaris (Pearsall's strain) was grown in pure culture in medium buffered at *p*H 6·1[4]. Glucose was added, when required, in a final concentration of 1·0 per cent. The cultures were shaken at 25° C. either in darkness or in continuous light provided by fluorescent tubes giving a light intensity of 900 foot-candles. Growth was measured by a turbidometric method which was checked by hæmocytometer counts. Growth was exponential over several days and the growth constant, k, was calculated from $k = 24(\log_{10} n_2 - \log_{10} n_1)/t_2 - t_1$ where n_1 and n_2 are the number of cells present after t_1 and t_2 hours growth respectively.

When cultures containing glucose were shaken in light, growth was inhibited by heavy water. Not only was the rate of exponential growth reduced but also the lag period was increased (Table 1). Attempts to increase the growth-rate in 90 per cent heavy water by adaptation were unsuccessful, unlike those of Moses *et al.*[3]. In our experiments the growth-rate in 90 per cent heavy water was the same after three sub-cultures as it was originally.

Table 1. Growth of *Chlorella vulgaris* in Different Concentrations of Heavy Water (D_2O)

Percentage D_2O (v/v)	Growth constant k	Approximate lag period (hr.)
0	0·70	20
50	0·46	20
70	0·35	150
90	0·20	160

The cultures contained 1·0 per cent glucose and were shaken at 25° C. in light of 900 foot-candles intensity.

In normal medium, *Chlorella* grows well in light without added glucose, and with our culture conditions, light stimulated growth even when glucose was added. However, when the medium contained 90 per cent heavy water, light inhibited growth; the cells grew fastest on glucose in darkness and illumination depressed the growth-rate. No autotrophic growth occurred at all (Table 2).

Table 2. Effect of Light on the Growth of *Chlorella vulgaris* in 90 per cent (v/v) Heavy Water (D_2O)

Glucose	−	+	+
Light	+	−	+
k—in normal medium	0·54	0·52	0·81
k—in medium with 90 per cent D_2O	0	0·23	0·17

The cultures were shaken at 25° C. The light intensity was 900 foot-candles. The concentration of glucose, when this was added, was 1·0 per cent.
The value of the growth constant, k, is given. Each value is the mean of two values obtained from two similar cultures.

Heavy water also had a marked effect on synthesis of chlorophyll. The illuminated cultures grown with glucose in 90 per cent heavy water were a bright orange colour. The pigments were extracted with ethanol and separated into carotenoids and chlorophylls. The absorption of the carotenoids was measured at 448 mμ and that of the chlorophylls at 660 mμ. These measurements indicated that the carotenoid content (per unit dry weight) of the orange cells was much the same as that of the normal cells but that the chlorophyll content was only one-fifth of normal.

Heavy water probably inhibits one of the dark reactions of photosynthesis[5,6]. In our experiments, the complete inhibition of autotrophic growth, which

is entirely dependent on photosynthesis, compared with the partial inhibition of heterotrophic growth, indicates that the photosynthetic mechanism is more sensitive to heavy water than many other reactions. Nevertheless, reactions other than the photosynthetic ones are inhibited, and the fact that illumination reduces the growth-rate of cultures growing on glucose in 90 per cent heavy water suggests that a substance produced by the photolysis of heavy water is more inhibitory than heavy water itself.

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Effect of Phosphorylated Methyl-naphthohydroquinones on the Response to Radiation in Barley Roots

It has been shown by Mitchell and Simon-Reuss[1-3] that some phosphorylated methyl-naphthohydroquinones are radiosensitizers of radiation-induced mitotic inhibition in chick fibroblast tissue. It seemed possible that these chemicals might be of value in the induction of mutations in plants as they afforded a means of modifying response to radiation. Accordingly, experiments were carried out to see if any of these compounds are radiosensitizers in plant tissues.

Tetra-sodium 2-methyl-1 : 4 naphthohydroquinone diphosphate (compound 1) and tetra-sodium 2 : 3 dimethyl-1 : 4 naphthohydroquinone diphosphate (compound 28), both known to be radiosensitizers in animal tissues, were used in these experiments. X-rays were provided by a 220 kV_p. 'Maximar' unit working at 15 m.amp., giving radiation of wavelength 0·22 A. A 1-mm. aluminium filter was used. The nominal dose-rate was 156 r./min. at a distance of 20 cm.

Seeds of barley (var. Earl) were germinated on filter paper moistened with distilled water, in Petri dishes in light at a temperature of 20 ± 2° C. Germination was apparent after 24 hr. and seeds were selected for uniformity at this stage. They were transferred to Petri dishes with filter paper moistened with 2 ml. of the chemical, and 18 hr. later irradiated as described above.

With doses less than 300 r., mitosis is at a minimum 3 hr. after irradiation, and root tips were fixed in glacial acetic acid at this time for studying mitotic inhibition. This was done by hydrolysing root tips in *N* hydrochloric acid at 60° C. for 12 min. and staining with Feulgen's reagent. Root tips were squashed and counts made of the number of cells in the different stages of mitosis. The seedlings were grown in the Petri dishes for up to five days after irradiation, and each day 50 seedlings were analysed for weight and length of root and shoot.

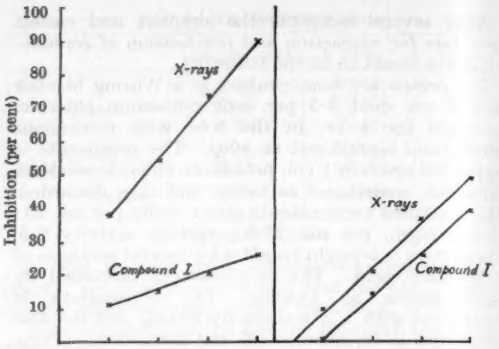

Fig. 1. Mitotic inhibition 3 hr. after irradiation

Fig. 2. Inhibition of increase of root fresh-weight 5 days after irradiation

Mitchell and Simon-Reuss[1] pointed out that to test for potentiation rigorously it is necessary to compare the effect of half-amounts of the two agents with the mean effect of the agents acting independently, and that there must also be a linear or sigmoid relation up to 50 per cent effect between response and dose for the separate agents. The effect measured with each of the agents acting alone should not be greater than 50 per cent. Figs. 1 and 2 show that there is a linear response to X-rays and compound 1 with mitotic inhibition 3 hr. after irradiation and with inhibition of weight of root five days after irradiation. Throughout these experiments the increases in weight and length of roots and shoots showed parallel responses to the treatments, so that only results for weights of roots are quoted.

Table 1 shows that compound 1 and X-rays have an additive effect on root growth five days after irradiation. Since growth is the product of many factors, it is not surprising that no potentiation can be demonstrated when measuring such a complex function. Five days after irradiation was chosen as the time to look for potentiation, as it is the earliest time at which the individual dose responses are linear. In fact, on the day after irradiation there is a stimulation of growth over the controls in all treatments, this effect gradually vanishing until on the fifth day the linear inhibition appears. Irrespective of the time after irradiation that root measurements were taken, no potentiation was observed. Compound 28 was not used for the experiments on growth measurements because of these difficulties of interpreting the results.

The examination of mitotic inhibition revealed the effects of the two chemicals and X-rays a short time after treatment, when responses should still be apparent and not masked as in the study of the increase of root weight and length several days after treatment. No measurements were made of chromosome abnormalities, but Bora[4] showed in broad bean roots that although these two chemicals do not cause chromosome breaks they increase the frequency of breaks induced by X-rays.

It is clear from Table 1 that compounds 1 and 28 have a radiosensitizing action on the mitotic inhibition induced by X-rays in barley roots. Both chemicals are themselves mitotic inhibitors, compound 28 being the more powerful. Compound 28 causes

Table 1

Treatment	Mitotic inhibition (per cent)	Potentiation factor	Treatment	Inhibition of fresh weight of root (per cent)	Potentiation factor
100 r. X-radiation	49·6		400 r.	49·1	
10^{-4} *M* Compound 1	26·7		10^{-3} *M* Compound 1	40·5	
5×10^{-5} *M* Compound 1	59·9	1·57 ± 0·16*	5×10^{-5} *M* Compound 1, followed after 18 hr. by 200 r.	46·1	1·03 ± 0·11
10^{-4} *M* Compound 28	38·8				
5×10^{-4} *M* Compound 28, followed after 18 hr. by 50 r. X-radiation	71·2	1·61 ± 0·18*			

*Significantly greater than 1 at 0·1 per cent level of probability. These figures are the means of five separate experiments.

metaphase accumulation, a finding which agrees with that of Mitchell and Simon-Reuss[2].

It appears from the above results that these two phosphorylated methyl-naphthohydroquinones act as radiosensitizers for mitotic inhibition in barley roots, and that this action is similar to that reported for chick fibroblasts in tissue culture and other animal tissues. Because they afford a means of modifying radiation response, they will be used to study the effect on X-ray induction of mutations in plants.

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Synthesis of Long-Chain Fatty Acids from 2-^{14}C-Acetate and ^{14}C-Glucose in *Trichoderma viride*

Although the synthesis of saturated fatty acids by the formal condensation of acetate units is well established, less attention has been paid to the formation of the common unsaturated acids (oleic, linoleic and linolenic) and their relationship to the saturated acids. The similarity in structure of oleic, linoleic and linolenic acids suggests a linked synthesis, and two processes have been considered: first a desaturation process leading from oleic via linoleic to linolenic acid, and secondly a hydrogenation process operating in the opposite direction. *Trichoderma viride*, a fungus which readily synthesizes palmitic, stearic, oleic, linoleic and linolenic acids, was chosen for studies with substrates labelled with carbon-14 in an attempt to obtain further information on the synthesis of unsaturated acids.

Mycelium was grown at 25° C. in a shaken 2 per cent glucose/acetate medium (*p*H 6·8), containing per litre: ammonium nitrate, 5·0 gm.; dihydrogen potassium phosphate, 3·5 gm.; crystalline magnesium sulphate, 2·5 gm.; ferric chloride, 0·08 gm.; zinc sulphate, 0·025 gm.; D(+)-glucose, 20·0 gm. and 5 ml. acetic acid neutralized with sodium hydroxide. After either two or four days growth the fungus was transferred to a single flask and equilibrated (4 hr.) with non-radioactive medium of similar salt composition containing, for the acetate experiments, 10 gm. D(+)-glucose and 0·25 gm. sodium acetate per litre, and for the glucose experiment 5 gm. D(+)-glucose and 0·125 gm. sodium acetate. At the end of this time 2-^{14}C-acetate or generally labelled ^{14}C-glucose was added, and after a suitable time the mycelium was removed by filtration and killed in boiling ethanol. Lipids were extracted in the usual way and the fatty acids obtained were separated by reversed-phase column chromatography[1], plated out and counted. Oleic and palmitic acids are not separable by this method, and the figure for palmitic acid was obtained from a second separation following removal of oleic acid after its conversion into short-chain acids by oxidation with alkaline permanganate. The figure for oleic acid was calculated by difference from that for the mixed oleic and palmitic acids.

The results in Table 1 show that radioactivity is incorporated into all the long-chain fatty acids within 3 hr. Closely similar results were obtained with 1- or 5-hr. periods. In every case the two saturated acids present (palmitic and stearic) and one of the unsaturated acids (oleic) had relatively high specific activities, whereas the other unsaturated acids (linoleic and linolenic) had much lower specific activities. This result obtained both in 2-day-old mycelium, which is rapidly synthesizing fatty acids, and in 4-day-old mycelium, in which fat synthesis is slow. Analysis of the free fatty acids (2·2 per cent of the total fatty acids), phosphatide fatty acids (41·4 per cent) and glyceride fatty acids (56·3 per cent) also showed the same distribution of radioactivity.

Table 1. Incorporation of Radioactivity into Long-Chain Fatty Acids after 3 hr. Growth on 2-^{14}C-Acetate or Generally Labelled ^{14}C-Glucose

Fatty acid	Age of mycelium and radioactive substrate					
	2-day glucose		2-day acetate		4-day acetate	
	c.p.m./ mgm.	gm./ 100 gm. fatty acid	c.p.m./ mgm.	gm./ 100 gm. fatty acid	c.p.m./ mgm.	gm./ 100 gm. fatty acid
Palmitic	508	20·9	9,628	20·9	1,129	21·3
Stearic	367	5·4	2,570	5·4	940	5·6
Oleic	329	29·0	7,509	29·0	701	24·0
Linoleic	53	42·3	1,522	42·3	149	44·0
Linolenic	65	2·2	434	2·2	48	5·0
Total c.p.m. supplied	$54·3\times10^6$		$17·4\times10^6$		$14·5\times10^6$	

These results indicate clearly that oleic acid is the first of the unsaturated acids to be formed and give little support to the hydrogenation hypothesis in which it is assumed that the most unsaturated acid is formed first. In view of the fact that similar results were obtained using either radioactive glucose or radioactive acetate as a carbon source it also seems unlikely that triose units are involved, as has been suggested, in the synthesis of unsaturated acids. A more probable explanation of the results would be that glucose is broken down into C_2 units before its incorporation into fatty acids.

In a further experiment 2-day-old mycelium, after being fed for 3 hr. with 2-^{14}C-acetate (see Table 1), was transferred, after careful washing, to a similar but non-radioactive medium for a further 2 days. During this time mycelium dry weight and lipid

weight approximately doubled. At 4 days the material was harvested, and the following specific activities (c.p.m./mgm.) were obtained: palmitic acid, 9,612; stearic acid, 2,924; oleic acid, 6,406; linoleic acid, 5,939; and linolenic acid, 2,309. None of the fatty acids showed any significant decrease in specific activity, and this is accounted for by the fact that there was a rise in the total count in the fatty acids from $44 \cdot 9 \times 10^4$ c.p.m. at 2 days to $72 \cdot 2 \times 10^4$ at 4 days. This could be the result of insufficient washing at the 2-day stage or it may be caused by the formation of a 'pool' of radioactive precursor within the tissue[2]. It is interesting, however, that whereas the specific activities of palmitic, stearic and oleic acids show only a small change, those of linoleic and linolenic acids have increased severalfold. This again indicates that these acids are formed later than oleic acid, and is consistent with synthesis by successive dehydrogenation of oleic acid, although other explanations cannot be dismissed on the present evidence. These results will be reported and discussed in detail elsewhere.

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Effects of Gibberellic Acid on Winter Pasture Production

The problem of restricted winter growth of pastures is of agronomic importance in many countries. This is especially true over large areas of Australia where fodder conservation, as one solution, is not economic. The possibility, inherent in the work of Morgan and Mees[1,2], Leben and Barton[3], Wittwer *et al.*[4,5] and Corns[6], that gibberellic acid might increase winter growth of continuously grazed pastures, does not appear to have been exploited beyond an initial experiment of Scurfield and Bull[7]. Results of that experiment were sufficiently encouraging to warrant our pursuing the matter further.

Experiments were carried out during the winter of 1958 on grazed pasture of *Phalaris tuberosa* and *Trifolium subterraneum*, sown twelve years previously. Four sites were chosen at random and, within each of these, four sub-sites. On each sub-site, sixteen plots (each of four sq. lk.) were located at random (subject only to the restriction that each had complete foliage cover). In each group of sixteen plots, four at random received nitrogen (as ammonium sulphate = 224 lb./acre), four an aqueous solution of gibberellic acid (= 3·69 oz./acre), four gibberellic acid plus nitrogen, and four were untreated (controls). Prior to treatment, all plots were clipped to ground-level. After treatment, they were clipped to this level on four occasions: the harvest data (lb. dry matter per acre) are given in Table 1.

Compared with controls, plots treated with gibberellic acid produced a greater yield of both grass and clover at harvests I and II, an equivalent yield of grass and a lesser yield of clover at harvest III, and a lesser yield of both at harvest IV. Over the four harvests so far taken, there was a significant increase in total yield of dry matter. This result confirmed that obtained previously[7], but not that of Morgan and Mees[1,2]. They found, after applying gibberellic acid (= 2 oz./acre) to pastures at various times from spring to autumn "in no case any net increase of yield over two or more cuts". More interesting, however, than an overall increase in yield was the stimulation of growth at a time of normally slow growth. Continuous meteorological records kept for the site showed that this alteration could be achieved under conditions of adequate rainfall and minimum temperatures as low as −9·5° C. (grass) and −4·5° C. (screen) (compare the records of Leben and Barton[3] and Wittwer and Bukovac[4]).

Changes in the botanical composition of the harvested material were of interest. Treatment with gibberellic acid caused a significant ($P < 0 \cdot 05$) depression of percentage clover in the material harvested on the second and third occasions. How far this was a direct effect of gibberellic acid and how far it reflected an altered competitive status of the species present was uncertain. The result failed to confirm that of Morgan and Mees[2], who found treatment with gibberellic acid to cause no change in the botanical composition of swards over a period between treatment and a third cut.

There were significant ($P < 0 \cdot 001$) reductions in percentage total nitrogen of *P. tuberosa* (from 4·25 to 3·66 at harvest I, 5·37 to 4·13 at harvest II and 4·57 to 4·20 at harvest III) as a result of gibberellic acid treatment. This confirmed earlier results[1-3,7] (not, however, those of Wittwer *et al.*[5]) obtained with other grasses. Despite such reductions, the total yield of nitrogen (Table 1) over all four cuts was significantly greater from plots treated with gib-

Table 1. Effects of Gibberellic Acid and Nitrogen on Dry Matter Production (lb./acre), Percentage Clover in Total Dry Matter and Yield of Nitrogen (lb./acre) from *Phalaris tuberosa*–*Trifolium subterraneum* Pasture
Mean of 64 plots at each harvest.

Treatment (applied July 10, 1958)	Harvest I (July 24, 1958)			Harvest II (Aug. 14, 1958)			Harvest III (Sept. 11, 1958)			Harvest IV (Oct. 16, 1958)			Total (4 harvests)	
	Grass	Clover	Clover (per cent)	Grass	Clover	Clover (per cent)	Grass	Clover	Clover (per cent)	Grass	Clover	Clover (per cent)	Dry matter	Yield of nitrogen
Control	82	9	10·2	176	51	22·5	308	136	30·6	890	834	48·4	2,486	92
+ Gibberellic acid	416*	68*	14·1	499*	102†	17·0‡	317	104‡	24·7‡	665*	538*	44·7	2,709†	98*
+ Nitrogen	68‡	7‡	9·8‡	226‡	42	15·5†	412*	101*	19·6*	1,042*	617†	37·2‡	2,515	—
+ Gibberellic acid + nitrogen	358*	35*	9·0	568*	63	10·0*	347‡	69*	16·6*	744*	417*	36·0‡	2,601	—

Degree of significance shown by: *$P < 0 \cdot 001$, †$P < 0 \cdot 01$, ‡$P < 0 \cdot 05$.

berellic acid than from control plots. Analyses have so far failed to demonstrate any similar effect of gibberellic acid on the nitrogen content of the clover.

The leaf 'burning' caused by the added ammonium sulphate makes the interpretation of the yield results from such plots somewhat uncertain, but evidently the quantitative effects of added nitrogen upon yield did not resemble those of gibberellic acid. Regarding the effects of nitrogen and gibberellic acid added together, no evidence of interaction between the two in relation to total yield was obtained. It was noted that both *Phalaris tuberosa* and *Trifolium subterraneum*, irrespective of simultaneous nitrogen application, showed chlorosis and other symptoms of gibberellic acid treatment[8,9], though by harvest IV chlorotic symptoms at least had disappeared.

An initial grazing experiment using Merino wethers on a similar pasture to the above has so far: (*a*) confirmed that treatment with gibberellic acid (=1·6 oz./acre) could increase late-winter pasture growth and (*b*) provided no evidence of a deleterious effect of herbage so treated on either the health or live weight of sheep after two months grazing. This latter fact supports the finding of Peck *et al.*[10] that gibberellic acid, administered in various ways directly to small animals, was non-toxic.

The results of the above experiments will be considered more fully elsewhere.

We are grateful to Mr. G. A. McIntyre for the statistical analysis of the data in Table 1.

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Geotropic and Morphological Alterations in Rice Seedlings caused by Plant Growth Regulators

During some investigations into the effects of gamma-BHC on rice seeds a number of experiments were carried out to test whether BHC was behaving in a similar way to plant growth substances in producing abnormalities. These experiments were carried out because it was found that gamma-BHC inhibited root growth at low concentrations (for example, in aqueous solutions at 10^{-5} *M*, root growth was reduced to half that of the controls) and because of a recent suggestion by MacLagan[1] that some of the chlorinated hydrocarbons, including BHC, might behave in a similar way to auxins in plant tissue. It has now been found (unpublished work) that BHC does not behave as an auxin so far as rice seedlings are concerned. Nevertheless, during these experiments some results were obtained which may shed some light on current theories of geotropism and also on the effects that auxins have on morphogenesis.

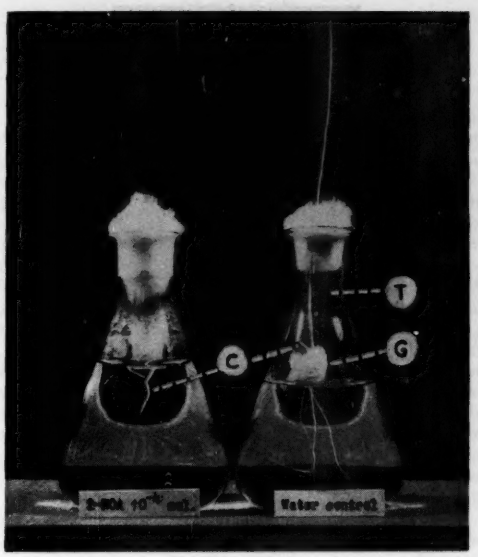

Fig. 1. Rice seedlings 7 days old growing in 10^{-4} *M* 2-naphthoxyacetic acid (left) compared with control (right). *C*, coleoptile. The apparatus consists of a 100-ml. conical flask containing 100 ml. of the test solution; the seed is held at the surface of the solution in a 10-mm. bore glass tube (*T*) the end of which is covered by cotton gauze (*G*); a cotton-wool plug holds the tube in position

Rice seedlings were germinated and grown for seven days in the apparatus shown in Fig. 1. The seeds were held at the surface of aqueous solutions of substances which have auxin activity and 'anti-auxins'; distilled water was used as control. Ten replicates were used for each treatment. Linear growth measurements and photographic records were made at the end of the experimental period. The substances with auxin activity were β-indolylacetic acid, naphthalene-1-acetic acid, methyl*iso*butylcarbonyl ester of 2,4,5-trichlorophenoxyacetic acid (2,4,5-T MIBC ester) (a crude preparation containing 71 per cent w/w) and 2-naphthoxyacetic acid. The substances used which are usually classed as anti-auxins were 1-naphthoxyacetic acid, 2,3,5-tri-iodobenzoic acid and α-(1-naphthylmethylsulphide)-propionic acid.

All the substances with auxin activity showed very similar effects on the seedlings though the concentrations necessary for the various effects differed. The three most obvious effects were inhibition of root growth, the production of an epicotyl (not present in the controls) and the reversal of shoot geotropism. The lowest concentrations required to produce an epicotyl and reverse shoot geotropism were as follows: 10^{-3} *M* indolylacetic acid, 10^{-4} *M* 2-naphthoxyacetic acid, 10^{-5} *M* naphthalene-1-acetic acid and 10^{-6} *M* 2,4,5-T MIBC ester. At these concentrations at least 60 per cent of the coleoptiles showed complete reversal of geotropism on all three occasions when these substances were tested. The epicotyls produced at these concentrations were 2–4 mm. long. In a large number of germination tests carried out in this laboratory on moistened filter paper or sand

Fig. 2. Photograph of 7-day old rice seedlings showing the reversal of root geotropism caused by 10^{-4} *M* 1-naphthoxyacetic acid. Note that secondary roots have the normal positive geotropic response

trays both in the light and in the dark there has never been any evidence of the production of an epicotyl. However, additional experiments have now been carried out in which rice seeds were placed in beakers and submerged beneath various depths of water from 3 to 10 cm.; under these germination conditions in the dark, epicotyls 4–6 mm. long were produced, but under the same conditions in diffuse daylight no epicotyls were found. Continuous aeration of the water in the case of submerged-germination tests in the dark decreased the size of the epicotyls to 2–4 mm. It would seem, therefore, that an epicotyl is normally produced under dark, poorly aerated conditions, but that high concentrations of substances with auxin activity are capable of inducing its formation even under light, well-aerated conditions. Microscopic examination of transverse sections showed that there were no distinguishable anatomical differences between epicotyls produced by the dark, poorly aerated conditions or by application of substances with auxin activity. This similarity would be expected if epicotyl formation is due to a rise in auxin concentration. Yamada[2] has shown that rice coleoptiles grow to a greater length under water than in the air, and has presented evidence which suggests that this is due to the reduced capacity of the tissues to destroy auxin when submerged; submersion, he found, was equivalent to a 20–30-fold increase in indolylacetic acid. The absence of an epicotyl in the light, even in submerged seedlings, would fit in with the demonstrated presence in plant tissues of the light-stimulated indolylacetic acid oxidizing system, originally shown by Galston[3] in etiolated pea epicotyls.

One of the most general effects of the substances with reputed anti-auxin activity was inhibition of root growth at and above the following concentrations: 10^{-5} *M* 1-naphthoxyacetic acid, 10^{-6} *M* 2,3,5-tri-iodobenzoic acid, and 10^{-5} *M* α-(1-naphthylmethylsulphide)-propionic acid. In this way they showed a similarity with the auxins, but unlike the experiments with auxins, there was never any evidence of an epicotyl being induced to form. Neither α-(1-naphthylmethylsulphide)-propionic acid nor 1-naphthoxyacetic acid reversed coleoptile geotropism, though 2,3,5-tri-iodobenzoic acid did cause a positive response in approximately 20 per cent of the seedlings at 10^{-6} and 10^{-7} *M*. The most interesting result was obtained with 10^{-4} *M* 1-naphthoxyacetic acid: when seeds were grown at the surface of a solution of this strength, 60 per cent or more of the seedlings showed a reversal of root geotropism, and the roots grew upwards into the air away from the solution (Fig. 2). In the cases where root geotropism was reversed, the roots were strongly inhibited and showed only 20–30 per cent growth compared with the controls. This reversal of root geotropism was obtained on all three occasions when this concentration of 1-naphthoxyacetic acid was tried.

These results are considered to support the classical theory of geotropism in which it is postulated that the shoot contains sub-optimal concentrations of auxin, whereas the roots contain optimal or supra-optimal concentrations. It follows that if the effective concentration of auxin in the shoot is raised to a supra-optimal level it should behave geotropically as a root; conversely, if the effective concentration of natural auxin in the root is reduced—for example, by an anti-auxin—to a sub-optimal level, then the root should behave as a shoot. It is interesting to note that out of the five 'anti-auxins' which Street[4] tested, 1-naphthoxyacetic acid was the most effective in counteracting the effect of a natural auxin which accumulates in excised tomato roots and causes 'ageing'. It may be significant, therefore, that 1-naphthoxyacetic acid was the only substance used in the present investigations which reversed the geotropism in rice roots.

These results will be discussed more fully in a later publication.

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Prolonging Dormancy in *Vitis vinifera* with Gibberellin

At Davis, California, grape buds developing on the basal portions of shoots normally enter a dormant stage about September. By the following April the buds usually begin rapid growth. Both a small amount of cold and a certain interval of time are required for dormancy to break. If the dormant buds could be made to grow in the autumn, the crop of the following year could be estimated. Since gibberellin has been reported to break the dormancy of buds in peach[1] and potato[2,3], and of epicotyls in the tree peony[4], it was tested for this purpose on grapes.

The potassium salt of gibberellic acid, supplied by Merck and Co., contained about 80 per cent active ingredient.

In one experiment, Zinfandel vines were sprayed with gibberellin at 0, 10, 50 or 250 p.p.m. on September 6, 1957, two vines per treatment. At this time the foliage was still green and the percentage of total

Table 1. Growth of Shoots on April 19, 1958, on Zinfandel Vines Sprayed the Previous Autumn with Gibberellin

Concentration of gibberellin (p.p.m.)	Average length per shoot (in.)	No. of shoots per vine on spurs	No. of water sprouts* per vine
0	3·0	18	56
10	1·7	14	34
50	1·7	3	8
250	1·6	1	2

* Water sprouts refer to shoots arising from wood more than one year old.

soluble solids of fruit was about 20. After the leaves had fallen, in November, buds in all treatments were still dormant. In January 1958 vines were pruned to 9–11 spurs, each bearing 2–3 buds[5]. On April 19, control shoots were about 3 in. long (Table 1). The number of shoots decreased with increasing concentration of gibberellin, indicating that dormancy had been prolonged by gibberellin. Results were comparable in an experiment with the Tokay variety.

In a second type of experiment, cuttings of Tokay canes about 16 in. long were taken from the vineyard on December 4, 1957, and placed in a greenhouse with their bases in solutions containing 0, 0·01, 0·1, 1, 10 or 100 p.p.m. of gibberellin. The solutions, about 3 in. deep, were changed each week. There were thirty cuttings per treatment. At later intervals the cuttings were examined to see whether buds had begun growth. A cutting was said to have initiated growth when green was visible on one or more buds of a cutting. The results (Table 2) show that the higher the concentration of gibberellin, the longer the development was delayed. With one exception, no buds began growth by February 20 on cuttings placed in gibberellin in the range 1–100 p.p.m. This experiment was repeated with other sets of cuttings after they had been stored at a temperature of about 32° F. for two, four or six weeks. The trends were similar.

Table 2. Number of Tokay Cuttings out of Thirty that Showed Bud Growth on Various Dates after being Placed in Solutions of Gibberellin on December 4, 1957

Concentration of gibberellin (p.p.m.)	Date of readings		
	Jan. 23, 1958	Feb. 6, 1958	Feb. 20, 1958
0	7	9	17
0·01	3	8	17
0·1	1	5	13
1	0	0	0
10	0	0	0
100	0	0	1

It is of interest that gibberellin prolongs dormancy of buds of *Vitis vinifera* despite the fact that growth of shoots is greatly stimulated[6]. Gibberellin may be of use for retarding bud break in the spring in regions of early frosts.

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BIOLOGY

Acquired Immunological Tolerance of Heterografts

Acquired immunological tolerance of heterologous cells is of much lower degree than tolerance of homologous cells[1]. In experimental embryonal parabiosis[2], a large quantity of powerful antigenic stimulus is administered; this acts for a relatively long time during the 'adaptive period' and usually results in the formation of cell chimeras. A relatively high degree of heterologous tolerance was sometimes obtained by this method, for a prolonged survival of heterografts has been found in turkey–chicken parabionts[1], and an interspecific blood chimera demonstrated[3].

The degree of tolerance to heterografts has now been studied in further turkey–chicken parabionts. In one turkey parabiont, a chicken skin graft survived for three months and grew feathers. No chicken erythrocytes were demonstrated during this period in the turkey's blood. It is possible that no erythrocyte chimera was formed in this parabiont, or that, if it was formed, it disappeared from causes other than the immune reaction.

Skin heterografts from two chickens were applied to one turkey parabiont with persistent erythrocyte chimerism at the age of one year. Neither of the donors was the partner in the embryonal parabiosis. The grafts survived for 80 days, when the recipient died. A skin homograft from a turkey had been applied at the same time. This also survived for the whole 80 days. In control turkeys, skin homografts survive for only 10–14 days. Gamma-globulins were demonstrated by paper electrophoresis in the serum of this parabiont; it was therefore not a case of agammaglobulinæmia. Moreover, a guinea-fowl heterograft was destroyed by the turkey in the normal time. Immunological reactivity against heterografts from other species was thus maintained.

This example of a high degree of heterologous tolerance shows that in tolerance between turkeys and chickens, the immune reaction against individual antigenic differences within the donor species is lost, as well as its response to the individual antigens of the recipient's own species.

We then studied the occurrence of interspecific blood chimerism in turkey–chicken parabionts. Agglutination by specific antiserum revealed the presence of turkey erythrocytes five weeks after hatching in six chicken parabionts out of eight, and in four seven weeks after hatching. In one chicken, turkey erythrocytes were present up to the tenth week after hatching. Taking into account the length of the life of avian erythrocytes[4], most of these cases cannot represent a mere survival of the partner's erythrocytes exchanged during embryonal parabiosis, but must involve a repopulation by the partner's transplanted hæmatopoietic cells. They are therefore interspecific erythrocyte chimeras.

In one turkey, chicken erythrocytes were demonstrated up to the time of the bird's death at the age of 14 months, and actually comprised the majority of its erythrocytes. Chicken erythrocytes were also demonstrated in a specimen of blood taken after death.

The question arises whether this may not have been a case of pseudochimerism, as observed in avian bird embryos after transplantation of adult spleen cells[5]. Transplantation of prospectively immunologically active cells from the chicken parabiont to the turkey partner might have occurred during embryonal

Table 1

Plasma taken :	Chicken plasma No.:	Erythrocytes of chicken No.: 728	734	882	883	885	886	2911	Titre of anti-turkey hæmagglutinins
Before immunization	2120	−/−	−/−	−/−	−/−	−/−	−/−	−/−	8
	2300	−/−	−/−	−/−	−/−	−/−	−/−	−/−	4
After immunization	2120*	+/+++	+/+++	+/+++	+/++	+/+++	+/+++	+/+++	128
	2300	−/−	−/−	−/−	−/−	−/−	−/−	−/−	64

Chick No. 2120 immunized with blood of chimeric turkey parabiont (age one year). Chick No. 2300 immunized with blood of control turkey. Citrated plasma used for agglutination reactions undiluted, if not stated otherwise. Isoagglutination read after 30 min./24 hr.; titre of anti-turkey hæmagglutinins determined after 30 min.
* Plasma diluted 1 : 10.

parabiosis. These cells could probably acquire tolerance to the host's antigens. Later in life, however, they might lose this tolerance and form antibodies against turkey agglutinogens, which would then combine with the host's erythrocytes. Agglutination of these sensitized turkey erythrocytes by antiserum against chicken blood would thus simulate the presence of chicken erythrocytes—a case of pseudochimerism. In order to exclude this possibility, chicken agglutinogens were demonstrated in the blood of this chimera by means of immunization. Chickens do not possess natural agglutinins and do not form them even when immunized with turkey blood. Immunization of chickens with the blood of the chimera, however, resulted in the formation of *iso*agglutinins which also agglutinated the erythrocytes of other chickens in a relatively high titre (Table 1). Individual chicken antigens must have been present in the blood of the chimera to induce the formation of these antibodies. The demonstration of chicken antigens thus showed that this was actually a case of prolonged interspecific chimerism.

Table 2

Serum	Titre of serum: Non-absorbed	Absorbed: With normal erythrocytes of turkey No. 41	of chicken No. 735	With erythrocytes released after agglutination with chicken isoimmune serum of turkey No. 10/11 (chimera)	of chicken No. 725
Against turkey erythrocytes	16	<4		16	
Against chicken erythrocytes	16		<4		<4

Plasma diluted 1 : 2 and absorbed with equal volume of 33 per cent erythrocyte suspension (final dilution 1 : 4).

Another question investigated was whether erythrocytes with *both* chicken and turkey antigens occurred in the turkey chimera, in addition to chicken and turkey erythrocytes. Erythrocytes of the chimera were exposed to antisera against chicken erythrocytes. Clumped erythrocytes were separated from free cells by differential centrifugation, thus separating erythrocytes with chicken agglutinogens from those without them. The clumps were then broken up mechanically. Although the cells were free in physiological saline, they agglutinated in any serum, including their own. It was thus an example of the agglutination of sensitized erythrocytes in a protein medium. An attempt was therefore made to demonstrate turkey agglutinogens in these cells by absorption of chicken serum against turkey blood. This, however, did not reduce the agglutinin titre (Table 2). Erythrocytes containing turkey and chicken agglutinogens simultaneously were thus not demonstrated in the blood of this chimera by the method used in the present experiment.

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Antibody Formation in Tissue Culture

While the production of antibodies by explanted tissues or cells from immunized animals is readily demonstrable, most attempts to initiate antibody formation *in vitro* have met with failure. Only recently has the original claim for success[1] been substantiated by the observations of Stevens and McKenna[2], who found that normal rabbit spleen produced antibody to bovine γ-globulin after exposure to this antigen *in vitro*, but only if either the rabbit or the spleen had previously been treated with *S. typhi* endotoxin.

This report describes the *in vitro* initiation and production of two different antibodies in cultivated lymph node cells of normal rats : one reactive with the bacteriophage *T2*, the other with hæmocyanin (busycon canaliculum). In order to induce the formation of the *T2* antibody, it was necessary first to expose the virus to the cells of a rat peritoneal exudate obtained 48 hr. after intraperitoneal injection of beef infusion broth[3]. These cells (90 per cent macrophages) were washed once with buffered saline and then were packed by centrifugation. The packed cells (approximately $1{\cdot}4 \times 10^8$) were suspended in 5 ml. buffered saline containing 10^9 *T2* phage. The mixture was incubated at 37° C. for 30 min. The cells were then sedimented by centrifugation at 1,000 r.p.m., washed twice with buffered saline, ground in a Ten Broeck homogenizer and passed through a Seitz filter.

2 ml. of this cell-free filtrate were distributed among eight Esmarch dishes which contained rat lymph node cells maintained in Trowell's synthetic medium[4]. Preparations of untreated bacteriophage in comparable dilution, and of suspensions of normal rat macrophages alone, were added to other lymph node cultures. The plates were incubated at 37° C. under a 4 per cent carbon dioxide atmosphere. The supernatant fluids from these cultures were removed after varying periods of time and to one volume of fluid was added one volume of saturated ammonium

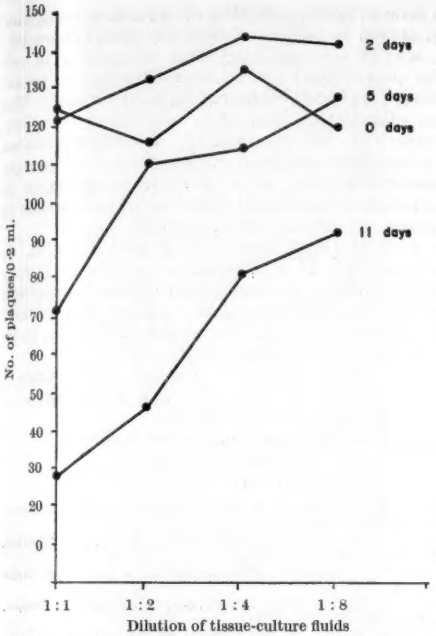

Fig. 1. Effect of length of incubation on the formation of *T*2 bacteriophage neutralizing antibody from rat lymph node cell tissue cultures exposed to rat macrophage-treated *T*2 bacteriophage

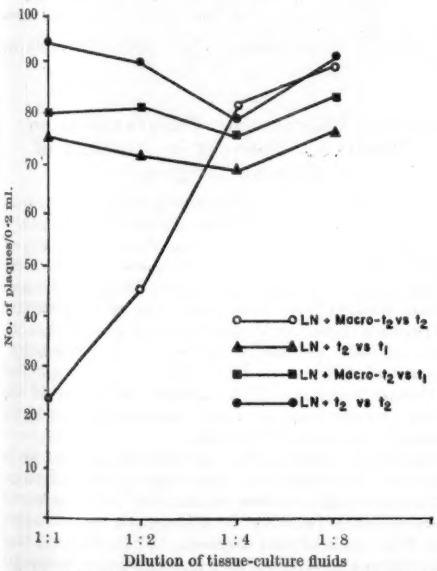

Fig. 2. Neutralization of *T*1 or *T*2 bacteriophage by fluids from rat lymph node cell tissue cultures exposed to either *T*2 bacteriophage or rat macrophage-treated *T*2 bacteriophage

sulphate. The resulting precipitate was dissolved in one-eighth the original volume and dialysed overnight against buffered saline of *p*H 7·6. These concentrated fluids were tested for phage neutralization by adding dilutions of the fluid to constant numbers of *T*2 phage, incubating for 30 min. at 37° C., plating on *E. coli B*, and counting plaques after 24 hr.

Phage neutralization was found only with fluids from lymph-node cells that had been exposed to macrophage-treated phage particles. As may be seen in Fig. 1, the neutralizing substance appeared on the fifth day and increased in titre to the eleventh day, after which the cells began to lose viability. Fluids which neutralized *T*2 did not neutralize *T*1 as shown in Fig. 2. The pretreatment of the virus by rat macrophages could not be replaced by treatment with rabbit macrophages or with HeLa cells. It was also found that the treatment of the phage with rat macrophages which had been damaged by freezing and thawing or by exposure to the Mickle vibrator was ineffective. However, a homogenate from rat macrophages prepared by the use of a Ten Broeck grinder could be substituted successfully for intact macrophages.

A primary antibody response to hæmocyanin was induced in lymph-node cells from normal rats by the addition to the culture of a cell-free filtrate from a mixture of hæmocyanin and rat macrophages (0·25 mgm. nitrogen/ml. hæmocyanin and approximately $1{\cdot}4 \times 10^8$ cells were used). Hæmocyanin alone again failed to elicit antibody formation. The titres of concentrates of tissue culture fluids, prepared in the same manner as above, are shown in Table 1. The hæmagglutination method of Boyden[5] was employed. If the tissue-culture fluids were freed of protein with perchloric acid, hæmagglutination activity was lost.

Table 1. HÆMAGGLUTINATION TITRES OF TISSUE CULTURE FLUIDS AGAINST HÆMOCYANIN

Post-incubation time (days)	Hæmagglutination titres Antigen added to rat lymph node tissue cultures	
	Hæmocyanin	Macrophage-treated hæmocyanin
0	0†	0
4	0	0
7	0	1 : 10
11	0	1 : 80–1 : 160

† Titre less than 1 : 10.

Further work is under way to characterize the *T*2 phage-neutralizing substance.

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Glutamine Metabolism of Normal and Malignant Cells cultivated in Synthetic Media

PREVIOUS communications from this Laboratory[1,2] have reported changes in the amino-acid content of synthetic medium *M* 150 during cultivation of a variety of freshly explanted tissues and have established a characteristic pattern of change with each tissue studied[3]. Cultivation of strain *L* (Earle) cells of mouse fibroblastic origin revealed a pattern of changes in the synthetic medium differing significantly from that of the freshly explanted tissues in regard to the metabolism of glutamine[4]. These studies have now been extended to a number of cell lines of malig-

Table 1. Changes in Glutamine Concentration of Synthetic Medium during Cultivation of Various Cells and Tissues

Tissue cultures	Change in glutamine content of medium*
Chick embryonic heart	+++
Chick embryonic kidney	++
Chick embryonic liver	++
Monkey kidney explants	+
Monkey kidney trypsinized	−
Strain *L* (mouse fibroblasts)	−−
HK 55 (human kidney)	−−
FL strain (human amnion)	−−
Strain HeLa (human malignant epithelium)	−−−
J-96 (human leukæmic)	−−−
J-111 (human leukæmic)	−−−
J-128 (human leukæmic)	−−−

* +, Increase −, decrease. Changes are based on most active period of metabolism of each culture type.

nant origin and appear to have established a consistent difference between the two types of cultures. All cultures of freshly explanted tissues so far examined accumulate glutamine in the culture medium, while all established cell lines studied utilize this amide from the surrounding fluid.

The composition of the synthetic medium and the methods of tissue cultivation have been described previously[1–5]. Samples of the culture medium were removed at appropriate intervals, pooled, and aliquots analysed by paper chromatography[1,2], using both one-dimensional and two-dimensional methods for separating completely the amino-acids of the culture medium. The results of these experiments are summarized in Table 1.

It is evident that the freshly explanted tissues show a marked increase in the glutamine content of the medium. On the other hand, the established cell strains, which are largely of human malignant origin, bring about a marked decrease in the glutamine concentration. This decrease is greatest with the HeLa cell strain and the three lines of human leukæmic cells, while the human kidney, human amnion, and strain *L* cells show the same change to a lesser degree. The behaviour of monkey kidney cells appears to depend on the method of preparation of the cultures. Primary cultures prepared from freshly explanted fragments cause a slight increase in glutamine content of the medium, while primary cultures prepared by trypsinizing the tissue to a cell suspension bring about a slight decrease in glutamine concentration. Similar variation in glutamine metabolism by monkey kidney cells cultivated in media supplemented with serum has been observed by Eagle *et al.*[6].

The completely synthetic medium used in these experiments supports the survival of freshly explanted tissues for more than 30 days and maintains the established cell strains for somewhat shorter periods. The results presented in Table 1 were obtained, therefore, under conditions of survival and active metabolism, but not of rapid propagation. It should also be noted that synthetic medium *M* 150 contains a high level of glutamic acid in addition to glutamine, but that the changes in glutamine content of the medium have been demonstrated previously[4] to be independent of glutamic acid.

The present observation that established cell strains, of predominantly malignant origin, reduce the glutamine content of the culture medium is in accord with the report by Roberts and Borges[7] that tumour cells are generally distinguishable from normal tissues by their low levels of free glutamine. It should also be pointed out that the present status of the established cell strains is somewhat uncertain. Those now available, whether of normal or malignant origin, appear to possess certain malignant characteristics, and it is considered that alterations in cell type or properties have occurred during the period preceding their establishment as cell lines[8,9]. The present observation that such cell lines can utilize glutamine may be interpreted, accordingly, either as a possible metabolic difference between normal and malignant cells, or as a possible alteration in cell metabolism associated with the establishment of permanent cell lines in tissue culture.

We wish to thank Dr. S. F. Kitchen, Dr. R. C. French, and Mr. L. F. Guerin, of the Virus Section of this Laboratory, for cultures of human kidney and human amnion and for monkey kidney tissue; and Dr. E. E. Osgood, of the University of Oregon, Portland, for the human leukæmic strains.

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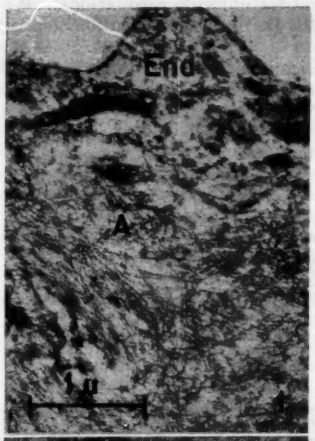
Electron Microscopic Observations on a Fibrous Component in Amyloid of Diverse Origins

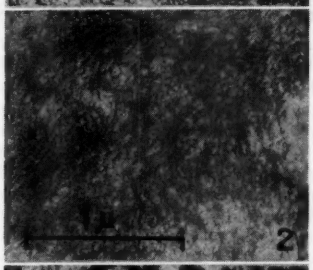
Amyloidosis in man and animals may be associated with other disease entities or may occur spontaneously. The relationship, if any, of the different forms of amyloid has proved difficult to ascertain, and the chemical nature of amyloid has not been established. Previous studies have indicated that it is predominantly protein in nature[1,2] and have suggested that the protein is not primarily collagen[3] or γ-globulin[4]. No chemical differences have been noted in amyloid of different sources. The precise definition of the amyloid protein has, however, been hampered by its insolubility in organic solvents.

Polarization microscopy in this laboratory as in others[5] has demonstrated that unstained and congo red stained accumulations of amyloid of a variety of sources exhibit positive birefringence with respect to the long axis of the deposit. This suggests that amyloid is not as structurally amorphous as it appears in the light microscope. To explore this possibility, electron microscopic studies were undertaken.

The following materials were used: (1) kidneys of 6 rabbits with casein-induced amyloidosis obtained immediately at the time of killing; (2) skin and subcutaneous tissue biopsy material from a patient with proved primary amyloidosis; (3) kidney of a patient with amyloidosis of parenchymal organs (liver, spleen, kidney) obtained 3 hr. post-mortem.

The tissues were immediately cut into specimens about 1 mm.^{3} and were fixed in both Dalton's solution





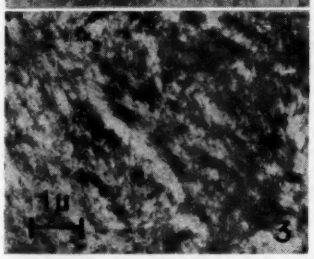


Fig. 1. Portion of rabbit glomerulus demonstrating amyloid fibrils in haphazard pattern. *A*, Amyloid; *End*, glomerular endothelial cytoplasm. (× *c.* 17,500)

Fig. 2. Portion of subcutaneous tissue biopsy of patient with primary systemic amyloidosis. Amyloid fibrils arranged in curved bundles. (× *c.* 23,500)

Fig. 3. Portion of renal tubular interstitial area of kidney obtained 3 hr. post-mortem. Fibrils in amyloid in swirls. (× 8,000)

and buffered osmium tetroxide in sucrose, then dehydrated in alcohol. Alternate specimens were put through 1 per cent phosphotungstic acid in absolute alcohol. The tissue was embedded in *n*-butyl methacrylate, and polymerized overnight with benzoyl peroxide at 60° C. Thin sections 250–500 A. thick were cut on a Porter–Blum microtome and examined in an *RCA-EMU-2B* electron microscope. Successive thick sections (up to 0·5μ thick) were cut from the same blocks and studied with the phase microscope; and, after staining, with the light microscope in order to determine the exact location of the amyloid. Formalin-fixed sections of the same material which had been prepared simultaneously with the osmium fixation were also cut, stained with hæmatoxylin and eosin, crystal violet, congo red and van Gieson dyes, to permit estimation of the amount of amyloid present. All three types of amyloid-containing tissues studied, bound congo red and exhibited metachromasia with crystal violet.

In the rabbit kidneys, the appearance of the amyloid was striking. It was localized between the basement membrane and the endothelial cell cytoplasm and in specimens fixed without phosphotungstic acid, had a fine granular appearance with delicate filaments running through it. In the material stained with phosphotungstic acid the filaments were more clearly defined and in several areas there was a suggestion of beading along the strands. The distribution of the filaments was either haphazard (Fig. 1) or, in areas near endothelial cell cytoplasm, in bundles. Although limitations imposed by sectioning prevented the precise delineation of fibril dimensions, they appeared to range in length from 1200 to 5000 A., and in width from 50 to 120 A.

The biopsy specimen of the patient with extensive primary amyloidosis also showed wavy bundles of delicate fibrils in the electron microscope. These correlated with the areas of amyloid as seen in the phase microscope and in stained sections. The dimensions were similar to those seen in the rabbit amyloid. The width varied from 70 to 140 A. and long strands up to 16,000 A. were measured. No cross-banding was apparent. These fibrils were often seen near collagen fibrils in the same section but were easily distinguished from them since the latter had a greater width and characteristic cross-banding. Fig. 2 demonstrates the delicate, wavy nature of the amyloid filaments as seen at a magnification of *c.* 23,500.

The amyloid in the kidney of the patient with parenchymatous involvement also demonstrated fine bundles of filaments similar to those noted above. Fig. 3 represents a 8,000-magnification of an area of amyloid in the renal interstitial area. The fine curved nature of the fibrils is again apparent.

Thus amyloid of diverse origin had similar submicroscopic structural characteristics. The fibrils as observed thus far appear to be morphologically distinct from collagen, thus corroborating previous chemical studies[2]. Further isolation and characterization studies of this fibrous element should contribute to our understanding of amyloid.

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Relationship between Salinity and Breeding of *Anopheles gambiae* in North-Eastern Tanganyika

A FEATURE of interest in the transmission of malaria in the Pare area of Tanganyika, before residual spraying, was the low sporozoite rate in *A. gambiae*, infections of less than 1 per cent normally occurring. Two factors thought to contribute to the low sporozoite-rate were facultative feeding on cattle, and a

low survival-rate in the vector[1,2]. A further possibility, that could not be excluded, was that the local strain of *A. gambiae* was also relatively resistant to infection or exhibited some innate difference in behaviour that reduced its chance of acquiring an infection, compared with strains in other areas. The existence of a low sporozoite-rate in salt-water *A. gambiae* on the humid coast of Tanganyika[3] gave rise to speculation as to whether the presence of saline soils in the Pare area was not associated there also with the low sporozoite-rate.

In the vicinity of rivers that descend from the South Pare Mountains, there are porous alluvial fans that are not saline. Surrounding these fans are much older impermeable soils from which rainfall and seepages from the mountains evaporate to a much greater extent, and are therefore saline. Salinification of the soils is particularly marked along the edges of fans where river seepages are held up by impermeable soils. 17 soil samples were obtained from different points along the valley between the South Pare and Usambara Mountains. The salinity of each sample was indicated by its conductivity in a 1–5 soil/water suspension at 25° C., and the results expressed as micromhos/cm. Conductivity of soil was 200 by a river, 950 to more than 3,000 in the swamp area, and more than 3,000 micromhos/cm. at the edge of an alluvial fan.

The chloride content of water in which *A. gambiae* was found breeding was estimated by the method of Sinton and Kehar[4], and the results expressed as parts of chloride per 100,000 of solution and as per cent sea-water chloride. Among 42 samples, the parts of chloride varied from 2·5 to 170 or, in terms of chloride concentration in sea water, a range of 0·13 per cent to 8·95 per cent. The distribution of saline breeding places coincided with that of saline soils. Parts of chloride were 2·5–5 near a river, 2·5–55 in the swamp area, and 20–170 at the edge of an alluvial fan.

Recently hatched larvæ from the eggs of 7 *A. gambiae* from huts, and 11 from outdoor box shelters, were subjected to Ribband's test[5] and none survived. Observations were then made, at 30-min. intervals for 8 hr., on mortality of recently hatched larvæ in a series of saline concentrations. The results (Table 1) show that the larvæ survived chloride concentrations equal to 30 per cent sea water for 8 hr., but died within 7½ hr. at 34 per cent.

Table 1. Mortality of *A. gambiae* Larvæ in a Range of Salinities

Parts Cl/100,000	1,450	1,050	850	800	650	550	525	500
Sea water (per cent)	77	55	45	42	34	30	28	26
Survival (hr.)	0·5	1·0	2·5	3·5	7·5	8·0	8·0	8·0

Ribbands[5] found that *A. melas* on the west coast could develop in 150 per cent sea water, whereas the maximum concentration in which freshwater *A. gambiae* underwent complete development was 37½ per cent sea water. Muirhead-Thomson[3] found evidence that salt-water *gambiae* at Dar es Salaam would not survive salinities higher than 83 per cent sea water, although they survived Ribbands's test. *A. gambiae* from Pare did not survive Ribbands's test or salinities higher than 34 per cent sea water. It is concluded that *A. gambiae* in the Pare area is typical freshwater *gambiae*, and that resemblances in sporozoite rate between *A. gambiae* in Pare, and salt-water *A. gambiae* on the east coast, are not related to the occurrence of saline soils in the former area.

We acknowledge with grateful thanks the help given us by Dr. T. E. Fletcher in providing apparatus and reagents for chloride estimations. We also owe thanks to Dr. D. Bagster Wilson for helpful criticism.

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Relationship between Epiphytic Algal Species and their Macrophytic Hosts

Epiphytes on the submerged parts of macrophytes have long been studied, but mainly from the taxonomic point of view. Little is known about the relationship of epiphytes with their host plants, or more particularly, whether certain species of algal epiphytes are associated with certain species of macrophytes. From observations made in Scotland, Sudan and in Malaya, I think that there is possibly some association. Mention was made of this in 1955[1], but since then there seems to have been no published information concerning this aspect of algal ecology.

Field observations are often marred by the fact that it is difficult to separate the effect due to decay from the natural epiphytic flora on healthy growing parts. The answer to such problems is to use healthy plants completely free from epiphytes, and to watch what develops on them in a particular environment. The difficulties in getting such 'clean' plants are innumerable. Dissected growing points are very slow to develop, while seeds of aquatic plants are often difficult to germinate. The use of algicides, such as copper sulphate and potassium permanganate, may not suppress fungi as well unless used in such quantities as may damage the growing points of the macrophytes. A solution to the problem was found by using 'Katadyned' water, which contains free silver ions in active form.

The plants chosen, *Enhydrias angustipetala*, *Naias graminea* and *Utricularia* sp., were all plants common in paddy swamps and in the fish ponds at this Station. Young healthy shoots were washed with a jet of water to free them as much as possible from attached algae, and each species was then placed in a large jar of 'Katadyned' water to which had been added some 'Sterasyl' or silver-coated gravel to maintain the content of silver ions in the water. The new young shoots which grew were completely free from epiphytes, and they in their turn were detached and transferred to fresh 'Katadyned' water. In this way healthy 'clean' plants were obtained.

A good length of the younger shoots of each species was transferred to a large fish pond, and sunk by weighting the lower end with a stone. Each plant was protected by a wire cage to exclude macrophyte-eating fish, but not smaller animals such as Crustacea, Rotifera and Protozoa. The *p*H of the water was 6·9. At the end of a fortnight the plants had increased in size and were coated with epiphytes.

They were carefully lifted, rinsed in water to free them from loose material, and then preserved in 5 per cent formalin.

A quick observation showed that, apart from some loose filaments of *Mougeotia capucina* (Bory) Agardh, the main epiphytic flora consisted of the diatoms *Gomphonema*, possibly *G. gracile* E. and *Eunotia*, probably *E. pectinalis* (Kz) Rbh. var. *elongata* V.H. and also attached plants of an undetermined species of *Oedogonium* (since no reproductive stages). As wide a selection of leaves as possible was taken, avoiding only the very youngest newly opened ones, which were still devoid of epiphytes. Twenty leaves from each species were examined; in the case of *Utricularia* a leaf consisting of several leaflets. The approximate area of each leaf was determined, and a systematic count of the epiphytes made. The results were expressed as density per sq. mm., the diatoms as cells, *Oedogonium* as attached whole plants. As was expected, the older leaves bore the heavier crops of epiphytes.

Table 1. Analysis of Densities of *Gomphonema*

	Total	Mean	Variance	
Macrophyte	Σx	$\bar{x}$	σ^2	$\log_e(\sigma^2)$
Utricularia	280·94	14·05	205·0380	5·32301
Naias	40·91	2·05	2·4217	0·88438
Enhydrias	36·60	1·83	2·2520	0·81182

From the results given in Table 1 it seems at first glance that the density of *Gomphonema* is much higher on *Utricularia* than on the other two species of macrophyte. Applying Bartlett's test for χ^2 it can be shown that this difference is significant.

$$\chi^2 = B/C, \text{ where } B = kn.\log_e S^2 - n\Sigma\log_e(\sigma^2)$$

and $C = 1 + (k + 1/3nk)$

Then $\chi^2 = 108·718/1·023 = 106·26$

For 2 degrees of freedom at the 5 per cent level $\chi^2 = 5·99$.

The three *Gomphonema* communities cannot be drawn from one and the same population.

Comparing only *Naias* and *Enhydrias* for *Gomphonema* we have $\chi^2 = 0·0496$. For one degree of freedom at 5 per cent level $\chi^2 = 3·84$. There is therefore no difference between the density of *Gomphonema* on *Naias* and *Enhydrias*.

No *Eunotia* was found on *Utricularia*, so only *Naias* and *Enhydrias* need be compared.

Table 2. Analysis of Densities of *Eunotia*

	Total	Mean	Variance	
Macrophyte	Σx	$\bar{x}$	σ^2	$\log_e(\sigma^2)$
Naias	95·37	4·77	147·9761	4·99721
Enhydrias	1·42	0·07	0·0234	−3·75502

$\chi^2 = 135·4$. For 1 degree of freedom at 5 per cent $\chi^2 = 3·84$. There is therefore a significant difference between the densities of *Eunotia* on *Enhydrias* and *Naias*.

Since no *Oedogonium* was found on *Utricularia* and only one specimen on all the leaves of *Naias* examined, it is obvious that there is a significantly higher population on *Enhydrias*.

The above results suggest therefore that *Enhydrias* favours the growth of *Oedogonium*, *Naias* that of *Eunotia*, while *Utricularia* bears the heaviest density of *Gomphonema*. These experiments were only preliminary and of short duration, and further experiments will be necessary. However, nothing is known about the physiological relationship, if any, between the host plants and the epiphytes, nor is it known what determines which species of epiphyte shall develop. Should the associations prove true, they must play a very important part in the productivity of waters. In the case of algal-feeding fish, such as *Tilapia mossambica*, the epiphytic flora may play a very important part in its diet; since there is evidence concerning the varying digestibility of the different groups of algae, any factor that may determine which species predominate may be of vital importance in the culture of the fish. It would therefore be of great interest to learn if any other similar experiments and observations have been carried out elsewhere.

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Improved Preservation of Tendon for Electron Microscopy through Wet Freeze-drying

Most of the information on the fine structure of biological tissues as revealed through electron microscopy has been gained from sections of material fixed with buffered osmium tetroxide, dehydrated in alcohols, and embedded in plastics. The degree of preservation of intracellular detail attained by such handling has been in general very consistent, and the preparatory techniques have been widely accepted as adequate. It has become evident in our recent work that, while some extracellular structures are also regularly well preserved, others seem to suffer considerable damage when processed in like fashion. This has especially been true in the case of tendon, where the component microfibrils appear to become widely separated (Fig. 1). The particular tendons under study are found in the leg of the domestic turkey, and they are of interest to us because they undergo calcification. It is obvious that in order to investigate the relationships between collagen fibrils and mineral crystallites tissue preservation is of the utmost importance.

In our efforts to determine whether or not the structural pattern shown in Fig. 1 represents a true picture of the fibrillar organization of intact tendon, specimens were subjected to different preparatory methods for purposes of comparison. One possibility which was explored arose from a purely physiological observation reported by Mueller and Szent-Györgyi[1]. They noted that when muscle fibres were dehydrated by immersion for three weeks in chilled acetone, transferred to cold ethyl chloride for one week, and then dried in air, they could still be made to contract after rehydration. This suggested that a remarkably high degree of structural preservation must have been attained, and led us to investigate the potentiality of this procedure as applied in histological technique. We have found that when similar treatment with acetone and ethyl chloride is substituted for the osmium tetroxide fixation and alcoholic dehydration steps or for the fixation step alone, sections from methacrylate-embedded tendon fibres present a quite different appearance under the electron microscope (Fig. 2). The collagen microfibrils are in close contact and form wide bands with a definite alignment of the 640 A. striations. The sheet-like configuration resembles that seen in preparations of both teased native collagen fibrils[2] and reconstituted collagen[3].

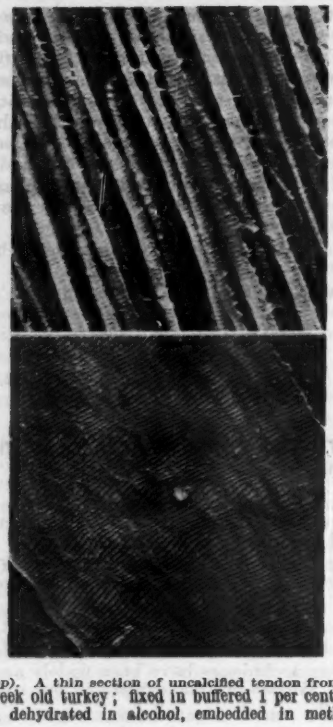

Fig. 1 (top). A thin section of uncalcified tendon from the leg of an 8-week old turkey; fixed in buffered 1 per cent osmium tetroxide, dehydrated in alcohol, embedded in methacrylate

Fig. 2 (below). A thin section of similar tendon which was simply treated with chilled acetone and ethyl chloride before embedding in methacrylate (both c. × 11,670)

We believe that this microscopic picture reflects a more intact state of tissue preservation, and more nearly represents the true structure of tendon fibrils than has hitherto been observed in sectioned material.

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BACTERIOLOGY

Bacterial Action in Pond-stored Logs

Extreme porosity was found in sapwood lumber produced from logs of *Pinus ponderosa* and *P. lambertiana* which had been stored in a pond from one to several months. The extreme porosity resulted in substantial over-absorption of water-repellent preservative solutions which are normally applied to millwork, with consequent difficulties in painting and utilization.

Investigation showed that bacteria operating under anaerobic conditions rapidly increased the permeability of the sapwood without substantially affecting strength. The attack was characterized by: (1) removal of parenchyma ray cell contents; (2) corrosion and eventual destruction of parenchyma, particularly in ray tissue. With the assistance of Dr. E. McCoy of the University of Wisconsin, at least one group of the destructive bacteria was identified as *Aerobacillus polymyxa* (flax retting bacteria) and related species. Unidentified bacteria in pond-stored logs have been previously reported by Stutz and Stout (Western Pine Association)[1] in connexion with brown-stain development in pine.

Many bactericidal and related treatments were applied to pond water. In preliminary laboratory tests the most economic and practicable controls found to date are acidification of infected water with sulphuric acid or application of sodium chloride.

Subsequent surveys indicate that this attack is widespread, at least in mill-ponds in California, but not generally recognized. There were some indications that causative bacteria may be present in sound logs before entry into the storage pond but attack was only found in ponded logs.

This work will be reported in detail in the *Journal of the Forest Products Research Society*.

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Cell-Wall Composition of *Leptotrichia* spp.

The status of the genus *Leptotrichia* has long been a matter of controversy, and in the current edition of Bergey's "Manual"[1] it is omitted completely. Nevertheless, there is no doubt of the existence in the oral flora of micro-organisms corresponding to the descriptions of *Leptotrichia*, and entirely distinct from other genera to which they have at various times been relegated by systematists. Two types are commonly found in the mouth, to both of which the name *L. buccalis* has been applied; these were distinguished by us as two species, *L. buccalis* and *L. dentium*[2]. Both are relatively large, filamentous, Gram-positive bacteria, but they differ considerably in morphology and metabolic characters, and the salient question arising from their recognition and distinction is whether they are sufficiently alike to be retained even in the same genus.

To the solution of this problem we have applied the technique of cell-wall analysis, according to the methods of Cummins and Harris[3], with certain modifications.

Sugars and amino-sugars were obtained from whole-cell hydrolysates, and analysed by descending, one-dimensional paper chromatograms using Whatman No. 1 paper and either *iso*propanol (160)/water (40) or phenol (80)/water (20) as solvents. Amino-acids were obtained from cell-wall fractions of cells broken by ultrasonic vibration, and chromatographed as for sugars, using *iso*propanol (70)/water (20)/acetic acid (10), or phenol (80)/water (20) + ammonia.

Leptotrichia buccalis and *L. dentium* yield exactly identical cell-wall amino-acid patterns, consisting of alanine, glutamic acid and DL- or DD-diaminopimelic acid as major components, and traces of glycine, serine, lysine and leucine. From our own comparative studies and the results of Cummins and Harris[4] this pattern appears to be representative of the genera *Nocardia*, *Mycobacterium* and *Corynebacterium*.

Sugar analyses showed arabinose and glucose as the major components of *L. dentium*, but only glucose in *L. buccalis*. Both gave small amounts of hexosamine (probably glucosamine) and ribose. The characteristic sugars of *Nocardia*, *Mycobacterium* and *Corynebacterium* are arabinose and galactose, which distinguish *L. dentium* from these genera. *L. buccalis* is even more clearly differentiated by its lack of arabinose.

From the evidence so far available it thus appears that the two species described by us[3] may reasonably be retained in the same genus, although it must be recognized that the taxonomic significance of amino-acid and sugar patterns in bacterial cell walls is yet poorly understood. There is already considerable variation in the literature regarding the taxonomic levels, that is, species, genera, etc., at which these patterns function as diagnostic characters. For this reason, the question of relationships between *Leptotrichia* and other groups still cannot be answered with any confidence. On the basis of morphology and cell-wall patterns, relationship to the *Nocardia*, *Mycobacterium*, *Corynebacterium* group of genera seems reasonable, whereas upon metabolic characters and cell-wall pattern, relationship with *Lactobacillus plantarum* is possible; it may be that this apparent divergence in relationships will be reconciled by future work. No relationship to *Fusobacterium* or *Actinomyces* is indicated by our studies of cell walls in these genera.

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Synthesis of Deoxyribonucleic Acid during the Division Cycle of Bacteria

In bacterial cultures undergoing synchronous growth, deoxyribonucleic acid has repeatedly been shown to be synthesized during part of the division cycle only (see review by Campbell, ref. 1). This could, however, be due to the physical or chemical operations used to induce synchronous division and need not reflect the normal pattern of synthesis of deoxyribonucleic acid. We have tested this possibility by examining autoradiograms of cells from uninfluenced cultures growing exponentially which had been exposed to thymidine labelled with tritium for short periods. Tritiated thymidine has already proved useful in studies of the mechanism of replication of deoxyribonucleic acid in bacteria[2]. The present experiments indicate that during normal exponential growth, synthesis of deoxyribonucleic acid is virtually continuous.

Two strains of *Escherichia coli* requiring thymine, namely, 15*T*⁻ (from the laboratory of Dr. S. S. Cohen) and *B*3 (from Dr. S. Brenner), were employed. These strains differ in morphology, mobility, rates of growth and sensitivity to penicillin. In addition, a wild-type strain of *Salmonella typhimurium* was used. Table 1 lists the media employed and the rates of growth obtained at 37° C. Tritiated thymidine (Schwarz Laboratories, Inc.) with a specific activity of 360 mc./m*M* was added to these media to a concentration of 3–5 μgm./ml. for *E. coli* and 12 μgm./ml. for *S. typhimurium*. Before addition of the labelled compound, exponential growth had been maintained at a constant rate for several generations in the presence of 0·5–1·0 μgm./ml. of unlabelled thymidine. Samples were removed at different times, treated with cold 0·5 *N* perchloric acid for 30 min., washed and resuspended in saline. This suspension was placed on agar blocks and, after the liquid had been completely imbibed, impression smears were made by touching the surface with 'subbed' glass slides (Ilford, Ltd.). The slides were covered with stripping film (autoradiographic film, *AR* 10, Kodak, Ltd.) according to the technique of Doniach and Pelc[3]. The time of contact with the film was selected to give an average of 3–5 grains per bacterium, and after development the bacteria were stained—through the film—with crystal violet.

Control experiments showed that the number of grains produced by the bacteria was reduced to the background value (about 1 grain/100μ²) after treatment with 100 μgm./ml. deoxyribonuclease for 30 min. at 37° C. Treatment with 0·5 *N* perchloric acid at 70° C. for 20 min. reduced the number of grains by 80–90 per cent, whereas the routine treatment with cold perchloric acid had no demonstrable effect. We therefore assume that nearly all the radioactivity which remains in the cells after extraction with cold acid resides in the deoxyribonucleic acid. Possible deoxyribonucleic acid-precursors of high molecular weight cannot be taken into consideration, since no chemical or genetic test has yet been developed which would distinguish such entities from deoxyribonucleic acid proper.

Table 1 summarizes nine experiments designed to show whether, during a fraction of the average generation time (see column 4), all or only part of the cells of a growing culture incorporate thymidine into their deoxyribonucleic acid. For each experiment the distribution of grains per cell was fitted to a Poisson distribution with the same mean value. This preliminary analysis revealed no systematic deviation from the Poisson distribution (see the *P* values of the last column), and it may therefore be

Table 1

Organism	Culture medium	Average generation time (min.)	Incorporation time as fraction of the average generation time	No. of cells counted	Average No. of grains per cell	Fraction of cells with zero grains	*P* per cent (of fit to Poisson distribution)
E. coli 15 *T*⁻	Glucose–salts–thymidine	45	0·1	301	3·36	0·047	20–30
E. coli 15 *T*⁻	*Glucose–salts–thymidine*	*45*	*0·2*	*240*	*2·65*	*0·083*	*80–90*
E. coli *B* 3	Glucose–salts–thymidine	48	0·1	257	2·58	0·112	5–10
E. coli *B* 3	Glucose–salts–thymidine	48	0·2	300	3·27	0·083	0·1–1
E. coli 15 *T*⁻	Casamino acids–salts–thymidine	28	0·2	292	3·52	0·058	0·1–1
E. coli 15 *T*⁻	*Rhamnose–salts–thymidine*	*64*	*0·1*	*283*	*3·03*	*0·060*	*5–10*
E. coli 15 *T*⁻	Rhamnose–salts–thymidine	64	0·2	140	4·87	0·007	60–70
S. typhimurium	Glucose–salts–thymidine	50	0·2	203	3·29	0·044	80–90
S. typhimurium	Glucose–salts–thymidine	50	0·2	213	4·05	0·009	60–70

concluded that roughly equal amounts of tritiated thymidine are taken up by individual cells during a short period of exposure.

A detailed analysis, to be published elsewhere, has led us to the conclusion that synthesis of deoxyribonucleic acid takes place over more than 80 per cent of the division cycle; it was not possible to decide whether the rate of synthesis remains constant or increases during the cycle. The model on which the analysis was based allows for the fact that some of the cells divide during the period of incorporation of labelled thymidine, and it could be shown that such sources of error as trackiness in the autoradiograms and variation in the individual generation times do not invalidate the interpretation.

Our results indicate that the pattern of synthesis of deoxyribonucleic acid found in synchronous growth experiments is artificial, and great caution must therefore be exerted in interpreting data from such experiments in terms of the normal division cycle. The rapidly growing bacterial cells apparently differ, with respect to their synthesis of deoxyribonucleic acid, from animal and plant cells, where this process seems to be restricted to part of the interphase period.

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Adaptation of Marine Bacteria to Growth in Media lacking Sodium Chloride

The adaptation of marine bacteria to the ability to grow on ordinary nutrient media prepared with distilled water instead of sea water has been observed in stock cultures; but, paradoxically, procedures designed to acclimatize these forms have not been successful[1,2]. The techniques previously reported have involved successive cultivation of the organisms in media progressively diluted with distilled water. A possible reason for the failure of these trials seems to be the complex nature of the adaptive changes required to allow for the simultaneous reduction or omission of all the components of sea water. Accordingly, the possibility of adapting marine bacteria to growing on media prepared with a synthetic sea water lacking only sodium chloride has been tested.

The cultures employed were isolated from samples of water from the Atlantic Ocean (St. Augustine, Florida) and the Gulf of Mexico (Cedar Key, Florida). All cultures required media prepared with sea water, and their cells lysed when suspended in distilled water. None of the organisms grew in media prepared with synthetic sea water from which sodium chloride was omitted; but on the other hand, sodium chloride alone failed to satisfy their mineral requirements. The synthetic sea water employed was compounded as follows: sodium chloride, 2·4 per cent; hydrated magnesium chloride, 0·53 per cent; hydrated magnesium sulphate, 0·70 per cent; potassium chloride, 0·07 per cent. The organisms grew adequately in media prepared with this salt mixture. The nutrient base in all media was 'Trypticase' (B.B.L.) 1·0 per cent, supplemented with 1·0 per cent fresh yeast infusion (10 per cent yeast). Media from which sodium chloride was omitted contained no added sodium ions, contained less chloride ions and had a lower tonicity; consequently even this single omission posed a complex problem in adaptation.

A number of attempts to adapt these marine cultures to non-dependence on sodium chloride by the dilution of growing cultures failed. These efforts were arranged to permit the optimum opportunity for the induction of the entire population to non-dependence on sodium chloride similar to the reported behaviour of *Escherichia coli* toward increased concentration of sodium chloride[3]. On the other hand, when heavy suspensions of the cultures were plated on the nutrient media prepared with artificial sea water from which sodium chloride had been omitted, a small proportion of the cells grew and produced colonies. These were capable of growing in subsequent culture in the absence of sodium chloride. When similar aliquots were plated on nutrient media prepared with fresh water, no colonies were obtained from any of the cultures tested. The frequency with which the cells independent of sodium ions occurred was compatible with their resulting from mutational events (Table 1). From each of the cultures a representative adapted colony was selected, purified by streaking and was maintained in stock on the medium free from sodium chloride.

Table 1. Incidence of Sodium Chloride-Independent Cell in Original Cultures and Stability of Adapted Strains

Culture	Incidence of sodium chloride-independent cells in unadapted cultures*	Percentage of adapted cells reverting after growth in sodium chloride†
I	$1·3 \times 10^{-9}$	68·0
II	$1·2 \times 10^{-8}$	0·0
III	$6·3 \times 10^{-9}$	99·2
IV	$3·4 \times 10^{-10}$	99·6

* Incidence is calculated as the ratio of the viable count obtained on the medium lacking sodium chloride to that obtained on the medium prepared with complete synthetic sea water.
† Values are the percentage of cells not capable of forming colonies in the absence of sodium chloride.

The adapted strains were osmotically less fragile than were the original cultures, although some lysis still occurred when the cells were suspended in distilled water. This residual fragility was not surprising since the medium retained a relatively high salt content despite the omission of sodium chloride. As would be expected, growth in media lacking sodium chloride was better with the adapted forms (Table 2). All the adapted strains were able to grow in media prepared with distilled water, although the

Table 2. Osmotic Fragility and Growth in Sodium Chloride-Free Media of Adapted and Unadapted Cultures

Culture	Per cent lysis in distilled water*		Growth in sodium chloride-free medium†	
	Unadapted	Adapted	Unadapted	Adapted
I	79	22	0·04	0·31
II	50	12	0·02	0·35
III	57	29	0·02	0·30
IV	56	28	0·01	0·35

* Per cent lysis is the percentage reduction in optical density resulting when a standardized heavy suspension in sea water is diluted 100 times in distilled water. The standard of comparison is a similar dilution in sea water.
† The values recorded are optical densities of the cultures after 24-hr. growth.
Optical densities were measured by means of a Bausch and Lomb 'Spectronic' 20.

initial colonies had apparently been unable to develop in such media. Thus it appeared that strains which had grown in the absence of sodium chloride had developed the additional capacity of growing in media prepared with distilled water. All the strains when grown in such media produced green or blue-green water-soluble pigments.

If the adaptation to non-dependence on sodium chloride in these isolates resulted from the selection of mutants, then logically the adapted character should be retained after being grown in the presence of sodium chloride. Accordingly, the adapted cells were grown in media prepared with sea water and were then tested for reversion. All but one of the cultures indicated some degree of reversion to the wild form, and in two cases it was quite marked (Table 1). This was reflected in the osmotic and growth properties of the cultures as well as a reduced percentage of cells independent of sodium chloride in the culture. These changes occurred during a single cultivation lasting 12 hr. The terminal cell population was about 10^{10} cells/ml. An obvious difficulty exists in the interpretation of these results, since the inclusion of sodium chloride in the medium was probably as rigorous a selecting force as was its omission.

The observations suggest, although by no means prove, that cultures of marine bacteria contain mutant individuals capable of developing in the absence of sodium chloride. Such cells are not able to initiate growth in the nutrient media prepared with distilled water, but require some other mineral component of sea water. For some reason as yet unexplained, the osmolysis of many marine bacteria usually does not occur in the presence of magnesium ions in the concentrations found in sea water[4] (that is, 0·05 *M*). As a possibility, it is suggested that such mutants growing in sea water media, although genotypically capable of growing in the absence of sea water, are phenotypically osmotically fragile. For their development in the absence of sodium chloride they must grow in the presence of magnesium ions to permit the initial maintenance of the cells. Once grown, such cells have the ability to develop in media prepared with distilled water. The testing of this hypothesis is planned.

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GENETICS

Homozygosity and *S* Gene Mutation

The *S* locus governing gametophytic self-incompatibility in plants is particularly suited to mutation studies. Pollen grains having an unmutated allele are unable to grow through a style carrying the same allele. In contrast 'mutant' grains produce pollen-tubes which may overcome the incompatibility barrier. Thus, mutation can be scored simply as seeds produced in normally sterile matings, and then subjected to confirmation by progeny phenotypes. Studies of *S* locus mutations have been confined to heterozygous plants[2-4] as homozygotes are difficult to obtain in many species. Since pollen *S* phenotype is a gametophytic character, it has been implied that mutation frequencies of alleles in homozygous plants would simply reflect those of alleles in the corresponding heterozygotes. The present study does not support this postulate.

Five *S* alleles (S_1, S_2...S_5) in *Petunia inflata* L. were assembled into the corresponding five homozygotes and ten heterozygotes at both $2n$- and $4n$-levels. Homozygotes are obtained readily in this self-incompatible species by self-pollination of young buds prior to *S* gene action in the style. Results will be reported for the five $2n$ homozygotes and three of the $2n$ heterozygotes—S_1S_2, S_1S_3 and S_2S_4. Two or more plants were used of each genotype, where possible, comprising a total of 16 different plants and their clonal propagants. X-rays were delivered to flowers and buds of pollen-parent plants at 250 kV., commonly with a filter of 1/4 mm. copper and 1 mm. aluminium. Pollinations were made exclusively with unirradiated flowers as females, since appreciable ovular damage occurred in flowers treated pre-meiotically (54 per cent abortion at 500 r., 88 per cent at 1,000 r.). Plants were grown and pollinations entirely made in bee-free control chambers at 22·5° C. Results are summarized as proportions of incompatibly pollinated flowers which produced seed-containing pods. Incompatible matings included both self- (for example, $S_1S_1 \times S_1S_1$) and cross-pollinations (for example, $S_1S_2 \times S_1S_1$).

A most impressive feature of the results was the differential response of homozygous and heterozygous clones (Table 1). Homozygous plants rarely set seed in incompatible matings (about 1 seed per 100,000 viable pollen grains), whether irradiated or untreated. In contrast the heterozygotes produced seed-containing pods in 6·8 per cent of the incompatible matings of untreated males and up to 42·0 per cent of those with X-rayed males (Table 1). No significant differences were observed among genotypes within either group. An average of 5·1 seeds per pod was obtained from the 286 selfed pods on treated and untreated heterozygotes, while compatible crosses on the same plants ranged from 180 to 240 seeds per pod. The proportions of seed-containing pods on heterozygous plants varied greatly depending on the time of treatment (Table 1). However, there was no significant variation with time among X-rayed homozygous flowers (heterogeneity $\chi^2 = 9{\cdot}2$, $P = 0{\cdot}30$). Meiosis occurred about ten days prior to anthesis (range 9–11 days).

Buds treated after meiosis did not differ significantly from the controls in mutation output, in accord with Lewis's observations[3]. The low *S* mutability of such post-meiotic buds on heterozygous

Table 1. Percentages of incompatibly pollinated flowers which produced seed-containing pods

	Heterozygotes		Homozygotes	
Days*	Percentage pod set	No. of flowers	Percentage pod set	No. of flowers
Control	6·8	147	0·8	250
< 6	6·1	82	1·4	140
7–8	10·7	131	3·2	62
9–10	22·2	265	0·5	211
11–12	32·0	256	0·8	255
13–14	42·0	157	0·8	239
15–16	26·4	140	0·5	200
17–18	10·0	70	1·7	182
19–20	18·2	77	0·0	82
> 21	6·4	78	0·0	100

* Number of days from X-irradiation to anthesis.

plants has been interpreted to indicate that S gene action occurred immediately following meiosis[2]. However, this low mutability in post-meiotic haploid cells would be accounted for if a major proportion of S mutation somehow depends on S allelic heterozygosity within the target cell. This sheds doubt on the validity of employing S mutation frequencies (in heterozygotes) to define the time of S gene action. An alternative argument is that S gene action in the pollen occurs as late as the second mitotic division—a timing which could account for the difference in place of inhibition of bi- and tri-nucleate grains[1].

The following mutation frequencies per million viable pollen grains were calculated from the data (11–12 days prior to anthesis is ±pre-meiotic interphase): 9·9, homozygote control; 21·4, homozygote —1,000 r. at 11–12 days; 84·0, heterozygote control; 1,320·0, heterozygote—1,000 r. at 11–12 days.

These values may serve as rough indices of mutation-rates. Variables influencing these calculations include: (*a*) the number of pollen grains, which ranged from 1,800 to 4,500 per pollination for the clones used; (*b*) pollen viability *in vitro*, which dropped to 46 per cent of control-rates in flowers treated between 9 and 16 days with 1,000 r. In addition: (*c*) seed sets averaged 3·0 for control pods, while pods from heterozygotes treated at 9 days or less averaged 3·0 seeds, from 9 to 14 days averaged 5·0 seeds and for greater than 14 days, 6·4 seeds. Finally (*d*) of the 36 'mutant' progeny grown approximately two-thirds have reacted as S mutants.

Twelve of these mutants studied have shown part inactivation of pollen, a phenomenon which led Lewis[3] to postulate that the S locus is bipartite with separable pollen and stylar units. One other mutant plant was of particular interest, for it reacted as if the mutant allele was a cross-over recombinant with the pollen activity of gene S_1 and the stylar activity of allele S_2.

These observations could be interpreted to suggest that the relatively high S mutability of heterozygous cells may occur as a result of genetic interchange. Equal crossing-over or gene conversion would be expected to produce mutants only in S heterozygotes, a significant possibility if the locus is a pseudoallelic aggregate or a composite of separable sub-units. Alternatively, if the S locus comprises synaptically equivalent stylar- and pollen-units, unequal crossing-over could lead to part mutation of pollen in heterozygotes by assembling on the same chromosome two alleles or pollen-units which interact by competition in the pollen (competition interaction leading to full self-fertility occurs in all ten heterogenic pollen classes of tetraploids derived from the heterozygous diploids studied). Unequal crossing-over also would produce partly deficient pollen gametes in both homozygous and heterozygous plants. It is hoped that a detailed investigation of mutant alleles will shed light on the relative significance of these events.

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Possibility of Assortive Mating in the Arctic Skua

Data have recently been collected in Caithness and Shetland on the numbers of the different mating types, dark × dark, dark × pale and pale × pale, of the colour phases of the Arctic skua, *Stercorarius parasiticus*. Although on Fair Isle intermediates, very similar to darks, are recognized; in the remainder of these data, this distinction is not made: for this reason, the intermediates in the Fair Isle data have likewise been added to the darks.

The observations at present available are given in Table 1, together with the date and names of collectors. The Fair Isle data were extracted directly from the record books there.

Table 1

	D × *D*	*D* × *P*	*P* × *P*
Fair Isle, 1954–58 (Williamson, K., and Davis, P. E.)	64	34	10
Foula, 1948 (Venables, L. S. V., and Venables, U. M. (ref. 1))	9	7	2
Unst, 1948 (Venables, L. S. V., and Venables, U. M. (ref. 1))	36	15	4
Yell and Fetlar, 1948 (Yeates, G. K. (ref. 2))	36	25	5
Noss, 1946 (Perry, R. (ref. 3))	21	9	7
Caithness, 1953 (Pennie, I. D. (ref. 4))	9	11	1
Totals	175	101	29

D, dark; *P*, pale

In the Caithness data, the single pale × pale mating was described as a pale × intermediate pair. The description of the intermediate, however, showed that this bird was in fact what would have been called a 'dusky' pale at Fair Isle. This means that the neck and belly are cream rather than white; but these matings have always produced apparently normal pale-phased chicks and are thus included with the pales: what are called 'intermediates' at Fair Isle lack the pale belly.

In the analysis given in Table 2, 2 × 2 contingency tests of independence have been employed: χ^2 for one degree of freedom with Yates's correction has been calculated.

Table 2

Locality	χ^2	Locality	χ^2
Fair Isle	1·99177	Yell and Fetlar	0·0078391
Foula	0·028800	Noss	5·04175
Unst	0·79781	Caithness	0·27320
	Total data	5·34064 (deviation χ^2)	

The χ^2 value calculated for all the data is thus found to be significant at just above the 2 per cent level. It is therefore important to test whether any significant heterogeneity is present. By summing all the separate χ^2 values, χ^2_{total} for 6 degrees of freedom is found to be 8·1412. The heterogeneity χ^2 is now obtained by subtracting $\chi^2_{deviation}$ from χ^2_{total}. We thus find,

$$\chi^2_{het.}\ (5 \text{ degrees of freedom}) = 2{\cdot}8005$$

This value clearly does not approach significance.

The conclusion to be reached from these results is that a strong possibility of a departure from random mating among the phases of the Arctic skua exists: a significant excess of like over unlike matings is present, thus indicating a tendency towards homogamous mating.

It is interesting to speculate on the advantages to be gained from this tendency towards homogamy. Fisher[5] says of homogamy in man: "Its principal biometric effects seem to be to increase the genetic variance produced by a given number of Mendelian factors with given gene ratios, and so to increase in

a fixed proportion the intensity of the selection to which each is exposed". In this sentence, Fisher is, of course, discussing quantitative variation. It would seem likely, however, that homogamy involving major genes will act in a similar manner by affecting the modifiers of these genes and thus increasing the variability of the expression, especially of the heterozygote. This must lead to a more rapid evolution of dominance: indeed, at Fair Isle, it has been noted that dark appears to be semi-dominant.

Fisher also suggests that the causes of homogamy are scarcely to be distinguished from those which, in sexual preference, produce direct selective effects. Sexual preference might have evolved directly, by the lessening of competition among the more variable families; but there also appears to be the possibility of the evolution of sexual preference by a form of sexual selection. On Darwin's theory of sexual selection in monogamous birds, Fisher[5] says: "He [Darwin] supposes in effect that there is a positive correlation in the females between the earliness with which they are ready to breed, and the numbers of offspring they rear, variations in both these variates being associated, as Darwin suggests, with a higher nutritional condition. Whether this is so in fact it is difficult to say, but it should be noted that the dates of the breeding phenomena of a species could only be stabilized if birds congenitally prone to breed early did not for this reason produce more offspring. The correlation required by Darwin's theory must be due solely to non-hereditary causes, such as chance variations of nutrition might supply. Whether or not there is such a correlation, it would seem no easy matter to demonstrate".

If the sexual preference in question were correlated with early breeding, then clearly the evolution of this preference would prove fairly rapid. On the other hand, if no such correlation were initially present, sexual selection might still result in the enhancement of the sexual preference. The early females must have a wide choice of mates not only in respect of preference for like-phased matings but also for characters like quality of display, physique, or other plumage characteristics. Thus, though it is true that if the proportion of females tending to homogamy is the same in late as in early breeding birds, then the same proportions in the phases of the males will be available to the late as well as the early females: yet, the males left for the late females may be deficient in other respects, thus leaving the late females with a more limited choice of mates; moreover, random fluctuations in the numbers of the different phases must further cut down the choice. This theory demands that there should be congenital variations in the sexual preference in relation to breeding time. It should be noted that in the Arctic skua, birds generally pair for life—divorces are fairly rare—and so this form of selection must be much slower than in a species in which new pairs are formed each year.

P. O'Donald

Department of Genetics,
University of Cambridge.
Feb. 14.

[1] Venables, L. S. V., and Venables, U. M., "Birds and Mammals of Shetland" (Edinburgh, 1955).
[2] Yeates, G. K., "Bird Haunts in Northern Britain" (London, 1948).
[3] Perry, R., "Shetland Sanctuary" (London, 1948).
[4] Pennie, I. D., *Brit. Birds*, **46**, 105 (1953).
[5] Fisher, R. A., "The Genetical Theory of Natural Selection", 1st and 2nd edit. (Oxford and New York, 1930 and 1958).

Recoverable Lethal Mutations in *Drosophila* Sperm

In an experiment with fractionated irradiation of *Drosophila melanogaster* males Lüning and Hannerz[1] showed that there was induced the same rate of chromosome breaks at anoxia as in air, but that there was a recovery after irradiation at anoxia which, however, could be blocked by a fraction of irradiation in air. Lüning and Söderström[2] extended these observations to sex-linked recessive lethals and concluded that there existed a similar recovery process among this type of mutations as among chromosome breaks.

Irradiation at anoxia induced changes which could either result in a recessive lethal mutation or reversion to a non-lethal state which could not be distinguished from the original state. If a second fraction of irradiation was given in air within 15 min. after the treatment at anoxia the recovery process was blocked and a rate of recessive lethals was obtained equal that when the whole of the dose was given in air.

In a paper presented to the second Geneva Conference on the Peaceful Uses of Atomic Energy, Lüning showed that the recovery of chromosome breaks could be blocked if the second fraction (in air) was given within 30 min. but not when given on later occasions.

From these results it was concluded that the recovery occurred between 30 and 40 min. after irradiation, and when it had recovered the subsequent irradiation in air had no influence.

A similar analysis using recessive lethals would be of great interest. First, the question arose whether there could be determined a time within which recovery occurred. The establishment of such a borderline should also give a proof of the existence of recoverable mutation processes. Secondly, it was desirable to know whether this borderline agreed with that obtained from chromosome breaks.

In order to study this, the conventional Muller–5 technique was used. 3–4 days old *cv:sc*^{8}*Y* males were given nitrogen for 15 min. before and during the treatment (5 min. = 2,750 r.). The males were then divided into four groups. After varying intervals, they were irradiated in air (3 min. = 1,650 r.). The intervals were chosen to test agreement with the recovery process in chromosome breaks, namely, 15, 30, 40 and 50 min. The males were mated for 6 hr. The females were then allowed to oviposit for two three-day periods. The procedure was then the conventional one. The criterion on lethality has been at least 12 *M5* males but no *cv* males. Some cultures were analysed also in F_2.

Four repeats were made which gave consistent results and for that reason are combined in Table 1.

As is seen from Table 1 the series falls into two groups with the borderline between 30- and 40-min. intervals, which agrees with that from chromosome breaks. Furthermore, the two series with intervals 15 and 30 min. are consistent and significantly higher than the series with 40 and 50 min. interval. This result is in agreement with the expectancy if the

Table 1

Interval	Recessive lethals	Tested X-chromosomes	Recessive lethals (per cent)
15 min.	222	2,154	10·3
30 min.	220	2,026	10·9
40 min.	161	2,049	7·9
50 min.	172	2,192	7·8

recovery of potential recessive lethals occurred between 30 and 40 min. after irradiation at anoxia. The establishment of this borderline may be taken as a proof that there may exist recoverable mutations, although it does not give any information about the ratio of mutations which has this possibility.

K. G. Lüning
H. O. Henriksson

Institute of Genetics,
University of Stockholm. Feb. 11.

[1] Lüning and Hannerz, *Hereditas*, **43**, 549 (1957).
[2] Lüning and Söderström, *Hereditas*, **43**, 563 (1957).

PSYCHOLOGY

Effects of Independent Variations in Stimulus and Response Probability

The time taken by human beings to react to one of a number of possible signals increases with the number of alternatives involved. The effects of jointly varying stimulus and response uncertainty have been long known and adequately discussed[1,2]. In the present investigation these were varied independently, by using situations where the same response was required for a number of stimuli. The number of responses, and the ratio of response/stimuli were varied.

Card sorting was used as a convenient task for a pilot study. The subject turned and dealt cards from packs held in the non-dominant hand, on to a number of piles. Each pile represented a group of stimuli to which the same response was required. The stimuli were letters and figures (72 point) stencilled on the cards in indian ink. Packs were made up for each condition, so as to vary the number of different symbols for each pile; the number of piles was of course constant for each condition.

Four conditions of response uncertainty were used, four packs for each condition being employed. In one condition packs were to be sorted into two piles, there being one, two, four and eight symbols per pile. Cards were sorted into letters, or figures.

The other conditions used four, six and eight piles, with response/stimulus ratios of 1/1, 1/2, 1/3 and 1/4 in each case. Ten subjects were tested on condition one, and a fresh batch of twelve on condition two. Four trials were given on each pack (that is, each response/stimulus ratio). A third batch of nine subjects was used on the six- and eight-pile conditions. Six of these had eight trials with each pack; the remaining three had sixteen trials with each pack. Sorting time was taken with a stop-watch. Packs were given in random order. For the first sortings in each condition, but never afterwards, indicator cards were attached to the boxes into which the cards had to be sorted. The order of the boxes from left to right was varied randomly for each subject. Subjects were students at a teachers' training college, and female shop assistants. No significant differences appeared between these groups.

The results of these tests are shown below. In condition one there was a marked significant increase in time between pack I and pack II. Times for packs II, III and IV did not differ. In condition two the results were similar. In condition three the most striking difference remains that between packs I and II. There is a non-significant difference between packs II and III, and a significant ($P < 0{\cdot}02$) increase between pack III and pack IV. In the fourth condition there are highly significant differences between all packs. The most striking is that between pack I and pack II. The results of the three practised subjects on the last conditions were of the same pattern.

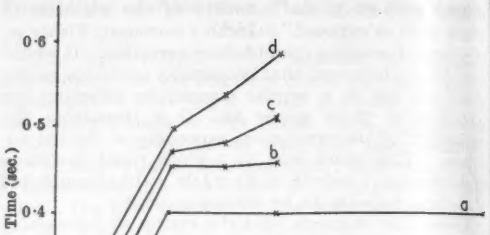

Fig. 1. *a*, 2-pile condition; *b*, 4-pile condition; *c*, 6-pile condition; *d*, 8-pile condition. Decision time/card was obtained by subtracting mean movement time, obtained individually for each subject, from the mean sorting time for each condition

In the last three conditions digits had been sorted into two of the piles. It was felt that the results might have been partly explained by the diminishing effect of the figures/letters dichotomy as the number of piles increased. Accordingly, the two- and four-pile conditions were repeated on six subjects practised in earlier trials using letters only. After eight trials on each pack in both conditions, the results showed the same pattern as the earlier tests. The increased practice necessary suggests that the figure/letter dichotomy had been used by the subject in earlier tests.

The results were still open to the interpretation that, despite the precaution of giving packs to the subjects face down, a small increase in reaction time with increase of stimuli/response was masked by subjects 'taking up the slack', and overlapping movement and decision-time to some extent. A choice reaction-time apparatus was used for investigating this, push buttons representing piles, and stimuli being projected on to a screen before the subject. Individual reaction-times were measured with a 'Dekatron' timer. Six subjects were used on the two-pile condition (letters/figures) and four on a four-pile condition (2 letters/figures, 2 letters only). The results gave the same pattern as the card sorting tests. In the four-pile condition considerable practice was necessary (500 presentations for each response/stimulus ratio) before this pattern emerged.

These results suggest that there are certain limits within which the response-time to groups of stimuli is dependent on the number of possible responses, rather than on the number of different alternative stimuli to which each response is to be made.

This work was carried out under a Department of Scientific and Industrial Research studentship, and the supervision of Mr. D. E. Broadbent.

P. M. A. Rabbitt

Psychological Laboratory,
University of Cambridge. Feb. 3.

[1] Quastler, H., "Information Theory in Psychology" (Illinois, 1955).
[2] Hick, W. E., *Quart. J. Exp. Psychol.*, **4**, 11 (1952).

FORTHCOMING EVENTS

*(Meetings marked with an asterisk * are open to the public)*

Monday, April 27

INSTITUTE OF ACTUARIES (in Staple Inn Hall, High Holborn, London, W.C.1), at 5 p.m.—Mr. S. Benjamin: "The Theory of Games and Its Application to the Rate of Interest".

ROYAL GEOGRAPHICAL SOCIETY (at 1 Kensington Gore, S.W.7), at 5 p.m.—Mr. E. M. Yates: "Dating the Cultural Landscape, with Examples Drawn from West Sussex".

INSTITUTION OF ELECTRICAL ENGINEERS, RADIO AND TELECOMMUNICATION SECTION (at Savoy Place, London, W.C.2), at 5.30 p.m.—Mr. G. F. Swann: "The Field Strengths Required for the Reception of Television in Bands I, III, IV and V".

UNIVERSITY COLLEGE (in the Anatomy Theatre, Gower Street, London, W.C.1), at 5.30 p.m.—Prof. O. Heckmann (Hamburg Observatory): "Newtonian Cosmology".*

UNIVERSITY OF LONDON (at Bedford College, Regent's Park, London, N.W.1), at 5.30 p.m.—Prof. J. Hämmerling (Max-Planck-Institut für Meeresbiologie, Wilhelmshaven): "Some Problems of Cell-Physiology with special reference to Studies on Acetabularia".* (Further lectures on April 28 and 29.)

ROYAL AERONAUTICAL SOCIETY (at the Institution of Civil Engineers, Great George Street, London, S.W.1), at 6 p.m.—Lecture on "The Electronics of Guided Missiles".

ROYAL SOCIETY OF ARTS (at John Adam Street, Adelphi, London, W.C.2), at 6 p.m.—Prof. J. J. Vincent: "Research in Cotton". (Second of three Cantor Lectures. Further lecture on May 4.)

Monday, April 27—Thursday, April 30

CORROSION EXHIBITION (at the Horticultural Hall, Westminster, London, S.W.1).*

Tuesday, April 28

INSTITUTION OF CHEMICAL ENGINEERS (at the Park Lane Hotel, Piccadilly, London, W.1) at 10.45 a.m.—Annual General Meeting.

UNIVERSITY OF LONDON (in the Large Lecture Theatre, Royal College of Science, Imperial Institute Road, London, S.W.7), at 5.30 p.m.—Prof. W. Lochte-Holtgreven (University of Kiel): "Light Emission Associated with Formation of Negative Ions". (First of three lectures on "Spectroscopic and Magnetic Studies of Thermally Excited Plasmas". Further lectures on May 1 and 4.)*

INSTITUTION OF MECHANICAL ENGINEERS, EDUCATION GROUP (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m.—Discussion on "Extended University Courses for the Better Graduate".

SOCIETY OF INSTRUMENT TECHNOLOGY (at Manson House, Portland Place, London, W.1), at 6 p.m.—Mr. M. E. F. Howarth and Mr. B. P. Watt: "Measurements in the Photographic Industry".

BRITISH INSTITUTION OF RADIO ENGINEERS, MEDICAL ELECTRONICS GROUP (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 6.30 p.m. Prof. G. Causey and Mr. R. S. Page: Two papers on "Electron Microphony".

SOCIETY FOR ANALYTICAL CHEMISTRY, PHYSICAL METHODS GROUP (at the Brunel College of Technology, Woodlands Avenue, Acton, London, W.3), from 6.30 p.m. to 9 p.m.—Demonstration of Laboratory-Made Equipment.

Wednesday, April 29

GEOLOGICAL SOCIETY OF LONDON (at Burlington House, Piccadilly, London, W.1), at 3 p.m.—Annual General Meeting. Dr. C. J. Stubblefield, F.R.S.: "Evolution in Trilobites" (Presidential Address).

UNIVERSITY COLLEGE (in the Anatomy Theatre, Gower Street, London, W.C.1), at 5.30 p.m.—Prof. O. Heckmann (Hamburg Observatory): "Some New Aspects of Relativistic Cosmology".*

CHALLENGER SOCIETY (at the Linnean Society, Burlington House, Piccadilly, London, W.1), at 5.45 p.m.—Annual General Meeting, followed by Dr. G. S. Carter: "The Conditions of Evolution in the Deep Seas".

ROYAL INSTITUTION (at 21 Albemarle Street, London, W.1), at 9 p.m.—Mr. G. de Q. Robin: "Antarctic".

Wednesday, April 29—Thursday, April 30

INSTITUTION OF ELECTRICAL ENGINEERS (in conjunction with the BRITISH NUCLEAR ENERGY CONFERENCE, at Savoy Place, London, W.C.2.—Convention on "Thermonuclear Processes".

Thursday, April 30

ROYAL SOCIETY (at Burlington House, Piccadilly, London, W.1), at 4.30 p.m.—Mr. R. L. F. Boyd and Mr. N. D. Twiddy: "Electron Energy Distributions in Plasmas"; Mr. R. L. F. Boyd and Mr. J. B. Thompson: "The Operation of Langmuir Probes in Electro-Negative Plasmas".

INSTITUTE OF INFORMATION SCIENTISTS (at the Berners Hotel, 10 Berners Street, London, W.1), at 6 p.m.—Symposium on "The Adaptability of Experience—an Inquiry into the Subject Qualifications Needed for Information Work". Chairman: Mr. L. J. H. Haylor. Speakers: Miss I. M. Slade, Mr. A. Gordon Foster, Mr. C. W. Hanson and Mr. F. Newby.

INSTITUTION OF ELECTRONICS (in the Assembly Hall of the University of London Institute of Education, Malet Street, London, W.C.1), at 7 p.m.—Mr. G. H. Byford: "Measurements in Aviation Physiology".

UNIVERSITY OF LONDON (at the Swanley Hall, Withersdane, Wye College, near Ashford, Kent), at 8.15 p.m.—Dr. I. A. Galloway: "Recent Advances in the Study and Control of Animal Virus Diseases".*

Friday, May 1

BRITISH PSYCHOLOGICAL SOCIETY, OCCUPATIONAL PSYCHOLOGY SECTION (in the Department of Psychology, Birkbeck College, Malet Street, London, W.C.1), at 1 p.m.—Mr. John Annett: "Problems of Training".

ANTIQUARIAN HOROLOGICAL SOCIETY (at the Science Museum, London, S.W.7), at 7 p.m.—Annual General Meeting.

Saturday, May 2

ASSOCIATION OF AGRICULTURE (at Senate House, University of London, W.C.1), at 10 a.m.—Conference on "Further Aspects of Animal Physiology".

APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the dates mentioned:

ASSISTANT LECTURER (with a degree in botany with some zoology) IN BIOLOGY—The Registrar, University College of North Staffordshire, Keele, Staffs (April 30).

ASSISTANT LECTURER (preferably with special interests in economic geography and statistics) IN THE DEPARTMENT OF GEOGRAPHY—The Registrar, The University, Leeds (May 1).

ASSISTANT LIBRARIAN (CHARTERED LIBRARIAN)—The Establishments Officer, Zoological Society of London, Regent's Park, London, N.W.1 (May 1).

LECTURER (with a good honours degree and preferably special qualifications in servo-mechanisms applied to electrical machines) IN ELECTRICAL ENGINEERING—The Registrar, King's College (University of London), Strand, London, W.C.2 (May 1).

ASSISTANT LECTURER (with a good honours degree in psychology and preferably some experience of teaching and research) IN THE DEPARTMENT OF PSYCHOLOGY—The Registrar, The University, Leeds 2 (May 2).

EXPERIMENTAL OFFICER or ASSISTANT EXPERIMENTAL OFFICER (with analytical experience) IN THE TECHNICAL CHEMISTRY DEPARTMENT, to work on problems connected with grass and milk—The Secretary, The Hannah Dairy Research Institute, Kirkhill, Ayr, Scotland (May 2).

LECTURER IN PHYSICS AND/OR CHEMISTRY—The Principal, Borough Road College, Isleworth, Middlesex (May 4).

SENIOR SCIENTIFIC OFFICER (with a science degree of a university in the United Kingdom with passes in biochemistry, zoology, botany, etc., and preferably five years experience in serology in the Health Service basic grade of Scientific Officer)—The Director, Regional Blood Transfusion Centre, Churchill Hospital, Headington, Oxford (May 2).

LECTURER IN PHYSIOLOGY—The Registrar, King's College (University of London), Strand, London, W.C.2 (May 4).

ASSISTANT LECTURER (with a good honours degree in zoology and special interests in parasitic protozoa) IN ZOOLOGY—The Registrar, King's College (University of London), Strand, London, W.C.2 (May 5).

LECTURER IN BIOCHEMISTRY—The Registrar, King's College (University of London), Strand, London, W.C.2 (May 7).

LECTURER (with a good honours degree and preferably a special interest in the field of numerical analysis) IN APPLIED MATHEMATICS—The Registrar, King's College (University of London), Strand, London, W.C.2 (May 7).

ASSISTANT LECTURER (preferably with a special interest in systematic botany) IN BOTANY, and a TUTORIAL ASSISTANT IN GEOLOGY—The Registrar, University College of Wales, Aberystwyth (May 8).

LECTURER or an ASSISTANT IN THE DEPARTMENT OF MATHEMATICS—The Secretary, The University, Aberdeen (May 9).

LECTURER or ASSISTANT LECTURER (VETERINARY SURGEON) IN VETERINARY PATHOLOGY—The Secretary, Royal Veterinary College (University of London), Royal College Street, London, N.W.1 (May 9).

RESEARCH FELLOW IN THE DEPARTMENT OF CHEMISTRY, for work on heterogeneous catalysis—Prof. C. Kemball, The Queen's University, Belfast (May 9).

SENIOR LECTURERS (with a good university degree in requisite subject, considerable postgraduate experience in research and/or teaching, and conversant with tropical work in the appropriate subject) IN (*a*) HORTICULTURE, (*b*) ANIMAL HUSBANDRY and (*c*) POULTRY HUSBANDRY at Kumasi College of Technology—The Council for Overseas Colleges, 12 Lincoln's Inn Fields, London, W.C.2 (May 11).

LECTURER IN EDUCATION, and ASSISTANT LECTURERS IN BIOCHEMISTRY AND PHYSIOLOGY—The Registrar, University College of South Wales and Monmouthshire, Cathays Park, Cardiff (May 14).

RESEARCH DEMONSTRATORS IN PHYSICS, ZOOLOGY, GEOLOGY and MINING; and a RESEARCH ASSISTANT IN ENGINEERING—The Registrar, University College of South Wales and Monmouthshire, Cathays Park, Cardiff (May 14).

LECTURER (preferably with qualifications in the comparative physiology of invertebrates), and an ASSISTANT IN THE NATURAL HISTORY DEPARTMENT—The Secretary, The University, Aberdeen (May 15).

RESEARCH ASSISTANT (PHYSICAL CHEMIST), for work with organometallic compounds—The Secretary, The University, Exeter (May 15).

RESEARCH FELLOWS (2) IN THE DEPARTMENT OF MEDICAL CHEMISTRY, John Curtin School of Medical Research, Australian National University—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (Australia, May 31).

BIOCHEMIST, Scientific Officer or Senior Scientific Officer grade (with a first- or second-class honours degree in biochemistry, and preferably postgraduate experience), to work on biological methods of preparing organic compounds labelled with radioactive isotopes—The Personnel Officer, Radiochemical Centre, U.K.A.E.A., Amersham, Bucks.

DEMONSTRATORS, INSTRUCTORS and ASSISTANT PROFESSORS IN THE DEPARTMENT OF PHYSICS, University of British Columbia, Vancouver—Dr. G. M. Shrum, c/o British Columbia House, 1–3 Lower Regent Street, London, S.W.1.

LABORATORY TECHNICIAN (with bacteriological or virological experience), for virus research—The Medical Superintendent, Common Cold Research Unit, Harvard Hospital, Salisbury, Wilts.

LECTURER (physicist with experience in X-ray crystallography, a knowledge of electronics and control mechanisms, and preferably previous experience in the rubber field) IN RUBBER PHYSICS—The Clerk to the Governors, National College of Rubber Technology, Northern Polytechnic, Holloway Road, London, N.7.

MASTER TO TEACH MATHEMATICS up to university scholarship standard—The Headmaster, Framlingham College, Framlingham, Suffolk.

MASTER TO TEACH PHYSICS to advanced level—The Headmaster, Malvern College, Worcestershire.

MATHEMATICS TUTOR (honours graduate in mathematics), to prepare to University of London degree standard—The Science Director, University Correspondence College, Burlington House, Cambridge.

RESEARCH PHYSICIST (preferably with experience in the use of ultra-high vacuum systems or in the chemical physics of surfaces) IN THE DEPARTMENT OF PHYSICS, for work which will involve electrical measurements applied to the study of surface contamination processes on semiconductors—The Professor of Physics, University of Reading, Physics Research Laboratories, Upper Redlands Road, Reading.

SCIENTIFIC ASSISTANT (ABSTRACTOR) (with a degree in agriculture or natural science, and a knowledge of languages)—The Director, Commonwealth Bureau of Pastures and Field Crops, Hurley, near Maidenhead, Berkshire.

SCIENTIFIC OFFICER (BOTANIST or AGRONOMIST), to work on the plant species and cultural conditions giving the best yields in the large-scale separation of leaf protein—The Secretary, Rothamsted Experimental Station, Harpenden, Herts.

REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

Great Britain and Ireland

National Institute of Agricultural Botany. Farmers Leaflet No. 8: Varieties of Winter Wheats. Pp. 4. Farmers Leaflet No. 9: Varieties of Spring Wheats. Pp. 2. Farmers Leaflet No. 10: Varieties of Barleys. Pp. 4. Farmers Leaflet No. 13: Varieties of Oats. Pp. 4. (Cambridge: National Institute of Agricultural Botany, 1959.) [192

Liverpool Observatory and Tidal Institute. Annual Report, 1958. Pp. 13. (Liverpool: Liverpool Observatory and Tidal Institute, 1959.) [192

Ministry of Housing and Local Government. Smoke is Your Enemy. Pp. 8. (London: Ministry of Housing and Local Government, 1959.) [192

Central Advisory Water Committee. Sub-Committee on The Growing Demand for Water—First Report. Pp. iv+28. 1*s.* 3*d.* net. Report of the Sub-Committee on Information on Water Resources. Pp. ii+20. 1*s.* 3*d.* net. (London: H.M. Stationery Office, 1959.) [192

Association of Universities of the British Commonwealth. Report of the Proceedings of the 1958 Home Universities Conference. (Conference convened by the Committee of Vice-Chancellors and Principals and held in the William Beveridge Hall, University of London Senate House, 12th and 13th December, 1958.) Pp. 120. (London: Association of Universities of the British Commonwealth, 1959.) 5*s.* [192

British Electrical and Allied Industries Research Association. Technical Report L/T362: Stability, Reignition Time and other Features of the Cold Arc. By A. E. Robson. Pp. 8+1 figure. 6*s.* Technical Report G/XT 164: Radiation Losses from the Arc Column. By Dr. H. Pelzer. Pp. 19+6 figures. 15*s.* (Leatherhead: British Electrical and Allied Industries Research Association, 1957 and 1958.) [192

Tobacco Manufacturers' Standing Committee. Research Papers, No. 3: The Constituents of Tobacco Smoke: an Annotated Bibliography. Edited by H. R. Bentley and E. G. N. Berry. Pp. ii+49. (London: Tobacco Manufacturers' Standing Committee, 1959.) [192

Parliamentary and Scientific Committee. Annual Report, 1958. Pp. 26. (London: Parliamentary and Scientific Committee, 1959.) [202

The Year Book of the Royal Society of London, 1959. (No. 63.) Pp. 287. (London: The Royal Society, 1959.) 21*s.* [202

London School of Hygiene and Tropical Medicine (University of London), incorporating the Ross Institute. Report on the work of the School for the year 1957–1958. Pp. 143. (London: London School of Hygiene and Tropical Medicine, 1959.) [202

Metropolitan-Vickers Electrical Co. Ltd. Metropolitan-Vickers Electron Microscope Wall Chart (Type EM6). (Manchester: Metropolitan-Vickers Electrical Co., Ltd., 1959.) [232

Eton College Natural History Society. Thirteenth Report, 1957–1958. Pp. 27+1 plate. (Eton: Eton College Natural History Society, 1959.) 2*s.* 6*d.* (Associates 1*s.*) [232

Agricultural Research Council. Postgraduate Studentships and Fellowships offered in 1959. Pp. 15. (London: Agricultural Research Council, 1959.) [232

Educational Research, Vol. 1, No. 2 (February, 1959). (A review for Teachers and all concerned with Progress in Education.) Pp. 80. Published in November, February and June. (London: Newnes Educational Publishing Co., Ltd., 1959. Published for the National Foundation for Educational Research in England and Wales.) 5*s.* 6*d.* per issue or 16*s.* 6*d.* per volume. [232

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